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Effect of Choline on the Estrous Cycle of the White Rat.

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Moore and Price[1] presented the hypothesis that the hypophyseal hormones excite gonadal production of germ cells and hormone secretion, and that the gonad hormones of either sex suppress pituitary secretion. This hypothesis is in agreement with the conclusions of Ihrke and D'Amour[2] that injections of male hormone concentrates from bull testis-tissue prepared by Gallagher and Koch caused a suppression of the estrous cycle of female rats as determined by the vaginal smear. This effect on the cycle, immediately abolished by the addition of pituitary substance, was explained by Ihrke and D'Amour on the basis of a diminution of pituitary secretions and a resulting suppression of the normal gametogenic and hormonal activities of the ovaries.

1 Moore, C. R., and Price, D., *Am. J. Anat.*, 1932, **50**, 13.
2 Ihrke, I. A., and D'Amour, F. E., *Am. J. Physiol.*, 1931, **96**, 289.

In 1937, however, Duncan, Gallagher and Koch[3] reported that crude male hormone preparations from testis tissue inhibit the normal estrous cycle in rats, but that this response is not due to the inhibitory action of the male hormone on the hypophysis, but solely to the phospholipin fraction free from male hormone. They further stated that crude choline obtained by hydrolyzing the phospholipins, and pure choline prepared synthetically likewise produced the same inhibition, but that pure testosterone in doses 20 times those found in crude testis-tissue concentrate did not inhibit the estrous cycle. The papers by Robson,[4] and Browman,[5] however, show that testosterone does cause a suppression of the estrous cycle in both mice and rats. The present work was undertaken in the attempt to confirm the report of Duncan, Gallagher and Koch[3] that synthetically prepared choline causes an inhibition of the estrous cycle of the white rat.

TABLE I.
Summary of Rats Used.

	Series A	Series B	Series C	Total
Choline injected rats	29	25	13	67
Uninjected controls	9	17	—	26
Saline injected controls	—	5	—	5
Total No. rats used	38	47	13	98

In Series A, vaginal smears were done daily on 38 rats from January 16th to February 17th, after which 20 of the rats were injected intraperitoneally with 4 mg. of acetylcholine chloride (Hoffmann-LaRoche) and 9 rats were injected intraperitoneally with 8 mg. of the same preparation on the following days, immediately after the smear was taken: Feb. 17th, 22nd, 23rd, 24th, 25th, 26th, 27th and March 1st and 2nd. Vaginal smears continued daily from March 3rd to March 19th showed no evidence of an inhibition of the estrous cycle. The rats were then injected intraperitoneally with 40 mg. (and in 9 expts. with 80 mg.) of choline chloride (Hoffmann-La Roche) on March 20th, 22nd, 26th, 27th, 28th, and 29th. No indication of an interference with the estrous cycle was found, although the experiment was continued to April 21st.

In Series B, after a control period of daily vaginal smears lasting from July 26th to August 19th, subcutaneous injections of 29 mg. (and in 2 expts. 59 mg.) of choline (from choline-HCl, Eastman

[3] Duncan, D. R. L., Gallagher, T. F., and Koch, F. C., *Science*, 1937, **85**, 23.
[4] Robson, J. M., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 49.
[5] Browman, L. G., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 205.

Kodak) were made on Aug. 19th, 20th, 21st, 22nd, 23rd, and 24th, immediately following the vaginal smear. This experiment lasted until Sept. 4th, yet no effect on the estrous cycle was noted. The rats in Series A and B were offspring of our active breeding colony, and littermate controls were used throughout.

In Series C, 13 adult rats with perfectly regular estrous cycles were selected from stock rats by a process of elimination and, after a preliminary control smear period of 16 to 44 days' duration, were all injected subcutaneously for 4 successive days (Dec. 15th to 18th) with 60 mg. of choline chloride (Hoffmann-La Roche) per 100 gm. body weight. The rats were then smeared daily for 17 days following the final injection. Even at this high dose-level none of the rats showed inhibition of the estrous cycle.

On the basis of 3 series of experiments on a total of 98 rats it is concluded, contrary to the findings of Duncan, Gallagher and Koch,[3] that estrous cycles in the rat are not modified by: (a) 4 or 8 mg. of acetylcholine chloride injected intraperitoneally, (b) 40 or 80 mg. of choline chloride injected intraperitoneally, (c) 29 or 58 mg. of choline injected subcutaneously, or (d) 60 mg. of choline chloride per 100 gm. body weight injected subcutaneously.

9830 P

Effect of Cysteine on Action of Insulin.

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An earlier paper[1] showed that alloxan produces hypoglycemia in normal rabbits. Alloxan is an oxidizing agent credited with special affinity for sulphydryl groups. If alloxan and insulin induce hypoglycemia through the same mechanism, and if alloxan's effect is produced through interaction with sulphydryl groups of tissues, then one might surmise that the normal hypoglycemic action of insulin could be diminished by the administration of sulphydryl compounds. Specifically, a quantity of sulphydryl groups equal in amount, reactivity and availability to those already in the tissues should diminish the hypoglycemic effect of a standard dose of insulin to one-half.

This paper presents the results of experiments in which cysteine

[1] Jacobs, H. R., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 407.

was given with insulin. Normal young adult rabbits were fasted for 24 hours before each experiment. Blood sugar determinations were made according to Miller and van Slyke.[2] The individual reactions to insulin in a dosage of 3 units per kg. subcutaneously were first established. (Fig. 1, a, b, c, d, e, f.) About one week later the same animals were given the same dosages of insulin, but, in addition, were given cysteine hydrochloride in neutralized solution subcutaneously separately from the insulin. The cysteine hydrochloride was given in 3 doses: 0.5 gm. 5 minutes before, 0.5 gm. 5 minutes after, and the remainder of the dose about 20 minutes after the insulin injection. The corresponding blood sugar curves are also represented in Fig. 1 (A, B, C, D, E, F). The dosages of cysteine given in the figures are those of cysteine itself, not the hydrochloride. Care was taken to prepare the cysteine solutions just before the injections, to minimize oxidation in air, and to insure neutrality.

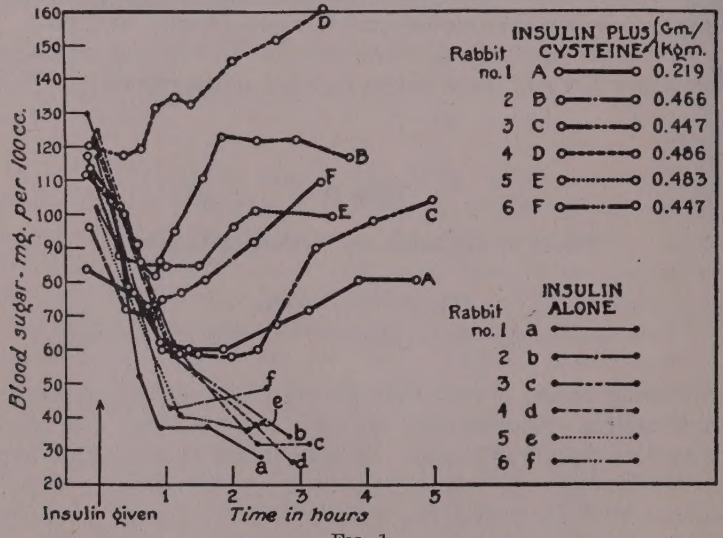

FIG. 1.
Blood sugar curves following insulin and insulin-plus-cysteine.

The effect of cysteine alone on the blood sugar level is shown in Fig. 2. The cysteine solutions were prepared and given in the same way as in the main experiment.

These experiments indicate that cysteine does actually diminish

[2] Miller, B. F., and van Slyke, D. D., *J. Biol. Chem.*, 1936, **114**, 583.

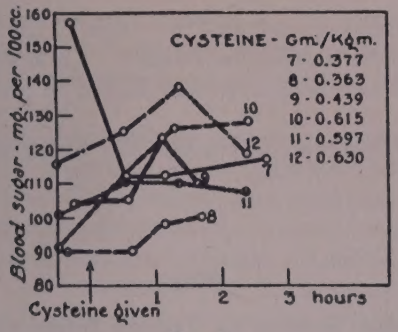

FIG. 2.
Blood sugar curves following cysteine alone.

the hypoglycemic effect of insulin. However, other amino-acids, such as glycine, alanine and glutamic acid[3,4] are known to cause hyperglycemia and to diminish the effect of insulin. Furthermore, aliphatic compounds such as propionic acid are convertible into carbohydrate.[5] Before the specific influence of sulphydryl compounds on the hypoglycemic effect of insulin can be defined a number of non-aliphatic non-amino sulphydryl compounds must be studied as well.

In vitro sulphydryl compounds such as cysteine, glutathione, thiolactic acid and thioglycollic acid all readily inactivate insulin.[6-9] That the effect *in vivo* is related to this type of inactivation seems unlikely.

In vitro Experiments. When cysteine hydrochloride and insulin are mixed in a phosphate buffer (pH 7.3) in concentrations of 0.1-0.2 mg. per cc. and 0.1-0.3 units per cc. respectively (final strengths) the following observations can be made: (1) In the absence of oxygen (Thunberg tubes) at 37°C. methylene blue is decolorized up to 50% faster by cysteine-plus-insulin than by cysteine alone. The decoloration is further hastened by some alcohols (ethyl, n-butyl) but is markedly retarded by aldehyde. Insulin alone has a very weak bleaching action. (2) In the presence of oxygen, the nitroprusside reaction disappears faster from the cysteine-plus-insulin solution than from the cysteine solution alone. The nitroprusside reaction is preserved if oxygen is excluded. Both

3 Nord, F., *Acta med. Scand.*, 1926, **65**, 1.

4 Pollak, L., *Biochem. Z.*, 1922, **127**, 120.

5 Ringer, A. I., *J. Biol. Chem.*, 1912, **12**, 511.

6 Freudenberg, K., and Wegman, T., *Z. Physiol. Chem.*, 1935, **233**, 159.

7 du Vigneaud, V., Fitch, A., Pekarek, E., and Lockwood, W. W., *J. Biol. Chem.*, 1931, **94**, 233.

8 Wintersteiner, O., *J. Biol. Chem.*, 1933, **102**, 473.

9 Stern, K. G., and White, A., *J. Biol. Chem.*, 1937, **117**, 95.

of these phenomena are explained by assigning the rôle of hydrogen transportation to the SH-insulin complex. These experiments, however, are inconclusive because insulin cannot be prepared chemically pure.

Conclusion. Cysteine diminishes the hypoglycemic action of insulin.

9831

Further Studies on Tongue Innervation.*

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This study constitutes an attempt to elicit proprioceptive impulses from the tongue musculature and to determine their pathway into the central nervous system. Since the cathode ray oscillograph has recently proven extremely valuable in the determination of the sensory innervation of the accessory musculature,[1] it was used in this study.

The observations of Langworthy[2, 3] (cat), Van der Sprenkel[4] (hedgehog), and Corbin, Lhamon and Petit[5] (monkey), suggest an afferent contribution to the hypoglossal nerve from the upper cervical dorsal root ganglia, proprioceptive in function. However, Hinsey and Corbin[6] obtained no evidence of myelinated fiber degeneration in the peripheral portion of the hypoglossal nerve of the cat after removing the upper 4 cervical dorsal root ganglia. Barron[7] obtained no evidence of proprioceptive activity in the hypoglossal nerve from traction on the tongue but did record action potentials, which he believed to be proprioceptive in origin, from the lingual nerve. Although Langworthy[1, 2] (cat) and Tarkhan[8] (rabbit) re-

* Aided by a grant from the Rockefeller Foundation.

† National Research Council Fellow in the Medical Sciences.

1 Corbin, K. B., and Harrison, F., *J. Comp. Neurol.*, in press.

2 Langworthy, O. R., *J. Comp. Neurol.*, 1924, **36**, 273.

3 Langworthy, O. R., *Johns Hopkins Hosp. Bull.*, 1924, **35**, 239.

4 Sprenkel, H. V. Van der, *J. Comp. Neurol.*, 1934, **36**, 219.

5 Corbin, K. B., Lhamon, W. T., and Petit, D. W., *J. Comp. Neurol.*, 1937, **66**, 405.

6 Hinsey, J. C., and Corbin, K. B., *J. Comp. Neurol.*, 1934, **60**, 37.

7 Barron, D. H., *Anat. Rec.*, 1936, **66**, 11.

8 Tarkhan, A. A., *Z. Anat. u. Entwickl.*, 1936, **105**, 349.

port the presence of muscle spindles in the tongue, Boeke[9] was unable to find muscle spindles in the tongue of the hedgehog, and we[10] failed to find muscle spindles in the intrinsic tongue muscles of the cat.

Sixteen cats under pentobarbital sodium anesthesia were used in these experiments. Recording leads, consisting of two 26 gauge silver electrodes chlorided electrically and separated by 1 to 3 mm. were connected to a 3-stage resistance coupled amplifier which led to a cathode ray tube and loud speaker. Amplification was such that an input of 10 uV gave a deflection on the screen of 1 cm. at the highest amplification used. The tongue was stretched by means of a thread through its tip. Twisting and distortion of the tongue were achieved by an insulated clamp applied to the tip.

In that portion of the hypoglossal distal to the ramus descendens hypoglossi there is little or no background activity of the type commonly seen in nerves mediating proprioceptive impulses from skeletal muscle.[1, 11] Stretching or twisting the tongue elicits no activity in this portion of the hypoglossal. When C1, C2 or C3 ventral rami are placed on the recording electrodes, there is background activity characteristic of proprioceptive fibers, but no increase in this activity is obtained by traction or torsion of the tongue. However, if the tongue is forcibly pulled so that the hyoid muscles are stretched or if these muscles are individually stretched, an increase in activity is obtained from C1 ventral ramus. The descending branch of the hypoglossal has been similarly studied but has never yielded a response to stretching of the tongue.

The lingual nerve, peripheral to its anastomosis with the chorda tympani, exhibits very little background activity. There is a burst of spikes when the upper surface of the tongue is lightly stroked. Similarly, an increase in the activity in this nerve is produced by pulling the tongue if such a maneuver causes the tongue to touch adjoining structures. However, if the tongue is stretched so that it does not rub against the teeth or oral mucous membrane, no increase in activity is obtained. Furthermore, brief application of 8% cocaine to the tongue's surface so as to just eliminate tactile impulses, abolishes background activity if it is present and no impulses arise when the tongue is stretched or twisted. These observations were made immediately upon disappearance of tactile responses, before

[9] Boeke, J., *Z. f. Mikr.-Anat. Forsch.*, 1927, **8**, 561.

[10] Lhamon, W., Yee, J., and Corbin, K. B., work in progress.

[11] Mathews, B. H. C., *J. Physiol.*, 1933, **78**, 1.

there was time for the penetration of cocaine into the muscles, as indicated by the fact that simultaneous faradic stimulation of the hypoglossal nerve evoked contraction of the intrinsic tongue muscles at the same threshold as before cocaine was applied.

The conclusion that proprioceptive impulses are set up in the lingual nerve by stretching or distortion of the tongue is not convincing unless tactile impulses are excluded. Barron's[7] statement that, "These discharges, when recorded in single fibre preparations, did not have as high an initial frequency as that described by Mathews ('33) for muscle spindles and the end organs producing them adapted more rapidly," suggests to us that these discharges may well have been tactile.

In 3 cats, C1 ventral ramus was sectioned on one side and C2 on the opposite side. After 9 days had elapsed, the distal portion of the hypoglossal was studied for degenerating fibers by the direct osmic acid technique,[1] which we prefer to the Marchi technique for peripheral nerves. No degenerating fibers were seen in these preparations after careful teasing and study.

From the data presented it is reasonable to conclude that if there are proprioceptive endings in the intrinsic muscles of the cat tongue (which seems very likely), the impulses set up by these endings are (1) so few in number as to escape detection by our methods; (2) mediated by fine myelinated or unmyelinated fibers which may not produce recordable potentials at the amplification employed; or (3), mediated by nerves other than those we have studied. If the proprioceptive impulses from the tongue musculature are mediated by unmyelinated fibers, unlike fibers generally accepted as proprioceptive in function, they would not have been detected by the degeneration technique employed.

Summary. The above data offer no evidence that the hypoglossal, lingual or upper cervical nerves mediate proprioceptive impulses from the intrinsic tongue musculature of the cat.

9832 P

Nicotinic Acid as an Essential Growth-Substance for Dysentery Bacilli.*

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Recent evidence has shown that nicotinic acid is a compound of considerable biologic importance. Current interest in this compound can be attributed primarily to the work of Warburg,[1] Euler[2] and their associates, which demonstrated that the amide of nicotinic acid is a constituent of the coenzyme from horse blood and the cozymase of yeast.

To those interested in the nutritive requirements of microörganisms this work has been of considerable significance as it supplied a clue to the chemical identity of one of the essential substances, or "growth-factors," needed for development of certain of the more exacting bacteria. In studies of *Staphylococcus aureus* Knight[3] demonstrated that a combination of nicotinic acid and vitamin B_1 (thiamin chloride) was effective in replacing a concentrate prepared from yeast. Neither substance would suffice in the absence of the other, but when both were supplied the staphylococcus developed in a culture-medium containing only known compounds. For growth of the diphtheria bacillus Mueller[4] found that nicotinic acid could replace one of several fractions obtained from liver. A combination of nicotinic acid, *beta*-alanine and, for some strains of the organism, pimelic acid was effective in promoting growth in the absence of tissue extract preparations.[5] Nicotinic acid without *beta*-alanine was relatively ineffective.[5]

The work reported here deals with the growth-promoting effect of nicotinic acid upon dysentery bacilli. It is of interest for two reasons: first, through the use of nicotinic acid it is possible to cultivate dysentery bacilli in a solution of known chemical com-

* The present investigation was aided by a grant to the University of Chicago from the Rockefeller Foundation.

1 Warburg, O., Christian, W., and Griese, A., *Biochem. Z.*, 1935, **279**, 143; **282**, 157.

2 Euler, H. v., Albers, H., and Schlenk, F., *Z. physiol. Chem.*, 1935, **234**, I; **237**, I; Euler, H. v., and Schlenk, F., *Z. physiol. Chem.*, 1937, **246**, 64.

3 Knight, B. C. J. G., *Biochem. J.*, 1937, **31**, 731.

4 Mueller, J. H., *J. Bact.*, 1937, **34**, 429.

5 Mueller, J. H., and Cohen, S., *J. Bact.*, 1937, **34**, 381.

pounds and second, nicotinic acid alone is strikingly effective without the addition of any other "accessory" factor.

The essential rôle of nicotinic acid was demonstrated by the use of a synthetic culture-medium consisting of 15 amino-acids,† dextrose, and several inorganic salts.[6] In such a medium many dysentery strains failed to grow. Upon the addition of nicotinic acid,‡ however, development of the organisms took place as indicated with the several representative cultures shown in Table I. With 0.1 microgram per cc. of medium all cultures developed promptly and in several of the tests the luxuriance of growth after 24 hours at 37°C. closely approached that observed in meat infusion broth. Amounts of 0.04 microgram were sufficient to support visible growth of all cultures within 24 hours, while 0.01 microgram or in some instances 0.004 or even 0.002 microgram sufficed for slower multiplication.

Preliminary tests with several derivatives of nicotinic acid were also made in a similar manner. Nicotinic acid amide showed activity in slightly higher dilution than that of the acid, methyl nicotinate

TABLE I.

Effect of Nicotinic Acid upon Development of Dysentery Bacilli in a Synthetic Medium.

Amount of nicotinic acid added, micrograms per cc. of medium	Flexner Development after days			Hiss Y Development after days			Strong Development after days		
	1	2	4	1	2	4	1	2	4
none	—	—	—	—	—	—	—	—	—
0.4	+++	+++	+++	++	++	++	+++	+++	+++
0.2	+++	+++	+++	++	++	++	+++	+++	+++
0.1	+++	+++	+++	++	++	++	+++	+++	+++
0.04	+	+++	+++	+	++	++	+++	+++	+++
0.02	—	+++	+++	—	+	++	++	+++	+++
0.01	—	?	+++	—	—	++	—	+	+++
0.004	—	—	+*	—	—	—	—	—	—*
0.002	—	—	—	—	—	—	—	—	—*

All tubes were inoculated lightly from suspensions of the respective organisms in a buffered inorganic salt solution, thus carrying over smaller numbers of cells than is the case when inoculations are made in the usual way directly from an agar slant.

— = no visible growth; + to +++ = light to very pronounced turbidity.

Observations were made at intervals after 4 days. In certain instances development of the cultures appeared later, or became more pronounced, upon continued incubation. These are shown by an *.

† The amino-acids were used as purchased without any additional purification.

[6] Koser, S. A., Finkle, R. D., Dorfman, A., Gordon, Mary V., and Saunders, F., *J. Inf. Dis.*, 1938, in press.

‡ Commercial samples of nicotinic acid were found to be impure and were subjected to a process of purification before use.

compared favorably with the acid while the ethyl ester was less active, requiring about 0.4 microgram per cc. of medium to exert growth-promoting effect.

Some additional observations have shown that different strains of dysentery bacilli exhibit different nutritional requirements. In a small collection of cultures an occasional strain was encountered that was able to develop in the synthetic medium without nicotinic acid. If this or a related compound is needed by these organisms they presumably must be able to synthesize it. Differences in amino-acid requirements became apparent on changing the composition of the synthetic medium. When a simpler medium containing asparagine, tryptophane, cystine, dextrose and inorganic salts was substituted for the more elaborate medium containing 15 amino-acids, some but not all of the dysentery strains refused to develop even in the presence of nicotinic acid. Evidently certain of the omitted amino-acids were needed by these cultures.

Several cultures of the Sonne type were also used. These developed to a certain extent in the synthetic medium without any added factor and produced a light but visible clouding which rarely became more pronounced with continued incubation. Upon the addition of nicotinic acid to the synthetic medium all of the Sonne dysentery cultures produced a much more luxuriant growth with pronounced turbidity within 24 hours.

In previous work[6, 7] the writers found that fractions prepared from spleen, liver, yeast, and other sources contained growth-promoting substances for dysentery bacilli. The same effect has more recently been observed with certain brands of gelatin,[8] and it has also been demonstrated that the activity for dysentery bacilli can be extracted with benzene from some gelatins. Amounts of benzene extract supplying 0.1 to 0.2 micrograms of total solids per cc. of medium supported visible growth of several strains of dysentery bacilli in a synthetic medium in which the organisms were unable to develop. Nicotinic acid alone is capable of replacing gelatin and the tissue-extract preparations. Whether the activity of our tissue fractions and of certain brands of gelatin is due to nicotinic acid itself is uncertain since the presence of nicotinic acid in these preparations has not been definitely determined.

[7] Koser, S. A., and Saunders, F., *J. Inf. Dis.*, 1935, **56**, 305; 1936, **58**, 121.
[8] Koser, S. A., Chinn, B. D., and Saunders, F., in press.

9833

Pressor and Other Effects of Antipyretics on Digitalis Action.

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The present investigation is concerned with the influence of antipyretics on digitalis. The antipyretics investigated were: the salicylates, acetanilid, phenacetin, aminopyrine, aspirin and antipyrine.

Dogs are quite sensitive and uniform in their reaction to digitalis. The average fatal dose of the tincture of digitalis, injected intravenously at the rate of 0.1 cc. per kilo body weight every 5 minutes, is 1.2 cc. per kilo, when dogs are anesthetized with 35 mg. of pentobarbital per kilo intraperitoneally. In previous work it was shown that high body temperature does not prevent the action of digitalis nor influence its toxicity. The variation from this fatal dose is surprisingly small. Therefore, the effect of the various antipyretics in changing the fatal dose of digitalis was tested.

In this report the point to which we wish to draw attention is the astounding effect on blood pressure which is effected by digitalis after all antipyretics. We select in this case antipyrine as the type.

I. Dog—23.2 kg., female; normal blood pressure 150 mm. Ten equal doses of 10% antipyrine were injected intravenously, making a total dose of 100 mg. per kg. The blood pressure after the antipyrine had been given was 130. At this point, digitalis was started in doses of 0.1 cc. per kg. The pressure rose gradually to 170 after 6 injections of digitalis. At this point the pressure rose sharply to 210, and, after another injection, it went to 300. The pressure was actually higher than this, as our manometer was incapable of recording a higher figure. After one more injection the dog died. The total dose of digitalis was 0.8 cc. per kg., as compared to a normal lethal dose of 1.2 cc. per kg.

This rise in pressure with a decrease in the fatal dose of digitalis was typical of all the antipyretics we have tried so far, when the antipyretic was followed by the digitalis. In cases where the 2 drugs are given simultaneously, the increase in pressure is usually not so marked, though it does occur. Thus, in 2 experiments in which the 2 drugs were given simultaneously (0.1 cc. per kg. of each at each injection), a rise of only 20 mm. was noted. However, the lethal dose in each case was only 7 injections, as compared to

9 in the first case. The amount of antipyrine used was thus less. Only 70 mg. per kg. were injected, as compared to 100 mg. per kg. in the first case.

It may be seen from these data that antipyrine markedly decreases the lethal dose of digitalis. More remarkable, however, is the extreme rise in blood pressure, which we have noted with other antipyretics also. Neither of these drugs alone produces a notable rise.

9834 P

Carotid Sinus Pressor Reflexes in Anoxia.*

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Whereas the rôle of the chemoreceptors in the carotid body and aorta for the blood pressure response to oxygen deficient gas mixtures was discussed in the preceding paper[1] the influence of the inhalation of low oxygen on the pressor reflexes will be presented in this paper. Gellhorn[2] reported that in the erect posture the blood pressure, after an initial rise, fell rapidly when the experimental subject inhaled 8% oxygen. Such a fall in blood pressure which readily leads to collapse was not seen when the subject inhaled the same gas mixture while in the recumbent position. These findings were explained by assuming a weakening of the pressor reflexes in anoxia. The following experiments were devised to test the validity of this hypothesis.

1. The influence of a change in posture on the blood pressure in the carotid artery was studied in narcotized dogs which were tilted from the horizontal to the vertical (feet down) position for 20 seconds. In a typical experiment the blood pressure fell about 40 mm. Hg. Hereafter 7% oxygen was inhaled and the same test was repeated. The fall of blood pressure increased to 70 mm. Hg.

* The experiments will be published *in extenso* in a monograph published by the University of Illinois Press.

† Aided by a grant from the Graduate School of the University of Illinois to E. Gellhorn.

1 Lambert, E., and Gellhorn, E., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 427.

2 Gellhorn, E., *Arch. Int. Med.*, 1937, **10**, 1267.

Upon readmission of air the values obtained successively were 60 mm., 50 mm., and 40 mm. Hg. respectively, indicating a complete reversibility of the effect of low oxygen.

2. A series of experiments was carried out in which the carotid sinus pressor reflexes were tested by raising the pressure in the carotid sinus. The procedure of Lim and Chang[3] was used. It was found that the fall of blood pressure resulting from an increase of pressure in the carotid sinus was diminished during the period of inhalation of 9% O_2. Upon readmission of air the original effects were reëstablished.

The experiments prove that the carotid sinus pressor reflexes are diminished under conditions of oxygen deficiency. This fact explains the different reactions of blood pressure in man to the inhalation of low oxygen gas mixtures in the erect and recumbent position.

Conclusions. During inhalation of 7-9% oxygen the pressor reflexes of the carotid sinus are greatly reduced. This is shown, (1) by a greater fall of blood pressure in the carotid artery upon changing from the horizontal to the vertical (feet down) position than is obtained under control conditions before and after the administration of low oxygen; (2) by a diminished response of the blood pressure under low oxygen to a given increase in pressure in the carotid sinus.

9835

Inactivation of Complement by Iodo-acetic Acid.*

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In preceding studies[1] it has been shown that normal guinea-pig complement can be reversibly inactivated by a number of oxidizing agents provided that the inactivation be controlled. The oxidants employed were iodine, H_2O_2, quinone, and O_2 and the reductants H_2S, KCN, ascorbic acid, and sodium hydrosulfite. It was also

[3] Lim, R. K. S., and Chang, H. C., *Chinese J. Physiol.*, 1936, **10**, 29.

* Aided by a Grant-in-aid, Division Medical Sciences, National Research Council.

[1] Ecker, E. E., Pillemer, L., Martiensen, E., and Wertheimer, D., *J. Biol. Chem.*, 1938, **123**, 351.

found that normal complement inactivated by controlled oxidation with the agents mentioned can be regenerated by heat-inactivated complements and by complement deprived of the 3d or 4th components.

Since it is known that iodoacetate combines readily with sulphydryl to form a mercaptide it was thought of interest to treat fresh guinea-pig complement with iodoacetate and to attempt a reactivation with various reductants and fractions of complement.

Fresh guinea-pig complement was diluted 1:10 with 0.85% saline and treated with varying amounts of a 0.5 M iodoacetate solution in bicarbonate-CO_2-buffer of pH 7.2. The mixtures were allowed to incubate for 15 minutes at 25°C. and thereafter immediately treated with various reductants.

The reagents and amounts employed are found in Tables I and II, and the method of complement-titration was the same as described by Ecker, Pillemer, Wertheimer and Gradis.[2] The results are given in percentages.

TABLE I.
The Reversible Inactivation of Complement by Iodoacetate.

	%
A Complement dilution 1:30 (control)	100
B 1 cc. of 1:10 complement + 0.45 cc. $CH_2ICOONa$ + 1.55 cc. saline	25
B + 1 mg. ascorbic acid	50
B + 3 " " "	66
B + 5 " " "	25
B + 10 " " "	0
B + 1 " glutathione (SH)	66
B + 3 " " "	66
B + 5 " " "	0

From these results it is evident that optimal amounts of ascorbic acid and glutathione (SH) markedly reactivated the iodoacetate-treated serum. No reactivation was observed when H_2S water (sat.) and N/100 KCN were employed.

TABLE II.
The Results Obtained with Various Components of Complement.

	%
A Complement dilution 1:30 (control)	100
B 1 cc. of 1:10 complement + 0.5 cc. $CH_2ICOONa$	33
1 cc. of B ; 1 cc. 1:10 inactivated complement (55°C. 30 minutes)	33
1 cc. of B + 1 cc. 1:10 NH_3-treated complement	33
1 cc. of B + 1 cc. 1:10 yeast-treated complement	100

[2] Ecker, E. E., Pillemer, L., Wertheimer, D., and Gradis, H., *J. Immunol.*, 1938, **34**, 19.

All these experiments have been duplicated.

Chow and Wong[3] have just reported the inactivation of complement by iodine, thus substantiating our previous findings.

Summary. Iodoacetate, a compound known to form mercaptides with sulphydryls, has been shown to inactivate guinea-pig complement. The inactivated complement was partially regenerated with ascorbic acid and glutathione (SH) but not with saturated H_2S water or KCN by our experimental procedure. This would indicate that the action of the iodoacetate on guinea-pig complement is not wholly upon thiol grouping, and that it may have other effects on the protein molecule. The fact that the iodoacetate-treated complement could be reactivated with two natural occurring reductants points to the possibility of a specific action of these two agents.

From these observations it must be assumed that iodoacetate acts primarily upon the protein and possibly on the 4th component of complement.

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Serum Lipoids and Complementary Activity.*

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Ottolenghi and Mori,[1] and Guggenheimer[2] showed that complement treated with ether became inactive after the solvents were allowed to evaporate. Toda and Misuse[3] reported later that the factor removed by extraction with chloroform or ether was the same as the 4th component. They further claimed that a similarity exists between the 4th component and the lipoid fraction since cadmium compounds, known to combine with lecithin, inactivate this component. Benzene inactivated their complement and the inactive serum was regenerated by the fraction soluble in benzene but not by the 4th component. Since this heat-stabile fraction did not reacti-

[3] Chow, Bacon F., and Wong, Sam C., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 120.

* Aided by a grant-in-aid, Division Medical Sciences, National Research Council.

[1] Ottolenghi, D., and Mori, M., *Centralbl. f. Bakt. I*, 1905, **38**, 338, 468.

[2] Guggenheimer, H., *Z. f. Immunitätsf.*, 1910, **8**, 295; 1911, **11**, 393.

[3] Toda, T., and Misuse, B., *ibid.*, 1933, **78**, 62.

vate ammonia-treated complement they postulated a hypothetical 5th component. Tokano,[4] however, could not demonstrate the existence of such a 5th factor but noted that ether or chloroform disturbed the 4th component. Hardy and Gardiner[5] removed lipoids from diphtheria antitoxin by means of alcohol and ether in the cold and showed that the lipoid-free globulin became soluble in saturated NaCl solution and that the antitoxic properties of the serum remained unaltered. Hartley[6] extracted lipoids by a similar method from rabbit antihorse, antihuman, antityphoid, and diphtheria-antitoxic serums, demonstrating a loss of precipitability of the extracted serums. Agglutination by the antityphoid serum and neutralization by the diphtheria antitoxin remained unchanged.

Horsfall and Goodner[7] more recently observed that the removal of lipoids from Type I antipneumococcal horse serum caused a loss of type-specific agglutination and precipitation, and that in the case of rabbit serum a marked reduction of these properties occurred.

In summing up, it may be stated that except for the work of Hardy and Gardiner, Hartley, and Horsfall and Goodner, little attention has been paid to the question of protein-denaturation during the process of lipoid-extraction. This is particularly emphasized in the problem of the relationship between lipoids and complementary activity.

The methods employed were fundamentally the same as advocated by Hartley, and Horsfall and Goodner except that the time of extraction was lengthened.

Method A. In this technic the spongy powder of 1 cc. active, dehydrated complement was extracted with 1 cc. cold absolute alcohol at −10°C. for a period of 24 hours. The dried complement was centrifuged and the supernate decanted. The residue was taken up in 10 cc. of cold absolute ether, agitated and kept at −10°C. for 48 hours, centrifuged and the supernate decanted. The residue was then dried *in vacuo* until free of all ether and dissolved in 1 cc. 0.85% saline.

Method B. The same procedure was followed except that the dried complement was shaken intermittently for 24 hours with cold absolute alcohol (kept at −10°C.). This was followed with cold petroleum ether for 24 hours and after removal of the petroleum ether the residue was again extracted with cold absolute ether for

[4] Tokano, Y., *ibid.*, 1936, **87**, 29.

[5] Hardy, W. B., and Gardiner, S., *J. Phys.*, 1910, **40**, 68.

[6] Hartley, P., *Brit. J. Exp. Path.*, 1925, **6**, 180.

[7] Horsfall, F. L., and Goodner, K., *J. Exp. Med.*, 1935, **62**, 485.

another 24 hours. The supernate was again decanted and the residue dried *in vacuo*.

Extractions were also made at 5°C. and at 20°C. Finally, the time of extraction was tripled (72 hours) for each solvent in both methods and at −10°C.

The phospholipid contents of the serums were determined as follows: The extractions were made according to the method of Bloor[8] with an alcohol-ether mixture. An aliquot of this was reduced to dryness in a pyrex test-tube and ashed with sulfuric acid and superoxol. The phosphates were then determined by the method of Fiske and Subarrow.[9] By these methods it was found that not more (usually less) than 50% of the phospholipids were extractable, indicating that the lipoids may be firmly bound to proteins or carbohydrates.

Fresh complement was further extracted by these methods but the complement was invariably inactivated.

Extractions of the same serums were also performed under anaërobic conditions but this did not prevent inactivation.

All complement titrations were made according to the method described by Ecker, Pillemer, Wertheimer and Gradis.[10]

Table I summarizes the results secured in a typical experiment.

TABLE I.
Initial Titers of Active, Dehydrated Complements Following Partial Removal of Phospholipids.

Methods	Temperature		
	−10°C.	5°C.	20°C.
Control	.03	.03	.03
Method A	.02	.03	—
" B	.02	.03	—

Even after prolonged extraction for a period of 9 days by methods A and B at −10°C. no change of titer occurred. The original titer of the dehydrated complement was 0.03 cc. of 1:30 and remained the same.

From these results it is evident that partial removal of the lipoids did not interfere with the activity of the complement. In fact, the removal of the lipoids often showed an improvement of initial titers which was strikingly noted in the case of an active dehydrated dog-complement with a normal initial titer of 0.05 cc.

8 Bloor, W. R., *J. Biol. Chem.*, 1928, **77**, 53.

9 Fiske, C. H., and Subarrow, Y., *ibid.*, 1925, **66**, 375.

10 Ecker, E. E., Pillemer, L., Wertheimer, D., and Gradis, H., *J. Immunol.*, 1938, **34**, 19.

of a 1:10 dilution. Following the method-B extractions the initial titer increased to 0.02 cc. of a 1:15 dilution.

The activity of active, dehydrated complement remained unimpaired after 7 days' extractions with chloroform, benzene and pyridine at a temperature of 4 to 5°C.

All the experiments were repeated with dog complement in the active, dehydrated state with no diminution of initial titers.

The recovered lipoids showed no hemolytic powers by themselves, and often exhibited anticomplementary properties when returned to the extracted residues prior to titrations.

Since it was shown by Ecker, Pillemer, Wertheimer and Gradis[10] that the initial complement-titers of guinea pigs, fed a vitamin-C deficient diet, were markedly lowered, it was considered of interest to compare the phospholipid contents of their serums and the serums of normal guinea pigs. The average phospholipid content of the serums of 50 normal guinea pigs, determined as phosphorus, was 2.14 mg. per 100 cc. of serum. The serums of 7 guinea pigs fed a vitamin-C deficient diet for 27 days gave an average of 2.04 mg. per 100 cc. of serum. This would seem to indicate that the serum phospholipids are not materially decreased during the course of vitamin-C deficiency. The initial complement-titers of vitamin-C deficient guinea pigs showed a decline of 75%, falling from 0.02 to 0.08 cc. of a 1:30 dilution. From these results it may be concluded that the phospholipid content of the serums plays no rôle in the reduction of initial complement-titers noted in the serums of guinea pigs fed a vitamin-C deficient diet.

Summary. Active, dehydrated complement showed no reduction of power when extracted in the cold with absolute alcohol, ether and petroleum ether. It was not possible to remove more than 50% of the total phospholipids of active, dehydrated complement by the methods described. Fresh complement was readily denatured by the solvents employed, namely, absolute alcohol, absolute ether, and petroleum ether. Treatment of active, dehydrated complement with chloroform, benzene and pyridine led to no change in initial titers. No evidence of a 5th component in the sense of Toda and Misuse was found. No relationship was found between the phospholipid contents of the serums of guinea pigs fed a vitamin-C deficient diet and their lowered complementary activities.

9837 P

The Capillary-Muscle Ratio in Normal and Hypertrophied Human Hearts.*

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In this investigation, the quantitative study of capillaries as initiated by Krogh[1] and by Wearn and collaborators[2, 3, 4] has been applied to normal and hypertrophied human hearts. The methods of injection and sectioning were essentially the same as those used on rabbit hearts in an earlier study from this laboratory.[4] Counts were made in 5 areas of each ventricle of the number of capillaries and fibers per square millimeter. Measurements were made to determine also the average diameter of the individual muscle fibers.

In this preliminary report we are able to compare the results of such a study in 10 normal and 10 hypertrophied human hearts. As compared with the normal hearts, the hypertrophied hearts show: (1) A marked increase in the average heart weight/body weight ratio and in the average heart weight, (2) a definite increase correspondingly in the average fiber diameter, and (3) a very evident decrease in the number of capillaries per square millimeter. At the same time, there is, (4) no change in the ratio of capillaries and

TABLE I.
A Comparison of Normal and Hypertrophied Human Hearts.

	Normal	Hypertrophied
No. of Hearts	10	10
Average Heart Weight (gm.)	289	650
" Ht. Wt./Body Wt. Ratio (x 10,000)	50.9	101
" Fiber Diameter (microns)	14	21
" " Area (sq. microns)	152	322
Capillaries per sq. mm. (average)	3992	2378
Fibers per capillary	1.4	1.3

* Presented at the joint meeting of the Experimental Medicine Section of the Cleveland Academy of Medicine and the Cleveland Section of the Society for Experimental Biology and Medicine, Cleveland, Ohio, October 15, 1937.

† Research Fellow in Medicine.

1 Krogh, A., *The Anatomy and Physiology of Capillaries,* New Haven, Yale University Press, 1922, pp. 5-11.

2 Wearn, J. T., *J. Exp. Med.,* 1928, **47**, 273.

3 Wearn, J. T., *Diseases of the Coronary Arteries and Cardiac Pain,* edited by R. L. Levy, New York, Macmillan Company, 1936, pp. 31-57.

4 Shipley, R. A., Shipley, L. J., and Wearn, J. T., *J. Exp. Med.,* 1937, **65**, 29.

fibers; that is, the capillaries do not multiply to accompany the increase in muscle mass.

From this study on human material, which is in accord with the experimental study of Shipley, Shipley and Wearn[4] on normal and hypertrophied rabbit hearts, it is evident that there is an increase in the distance from the center of a given myocardial capillary to the periphery of its region of supply, which is approximately proportional to the degree of cardiac hypertrophy. At the present time the oxygen utilization of normal and hypertrophied hearts is being investigated to determine whether or not the diminished anatomical vascularization of hypertrophied hearts results in a physiological impairment to the exchange of metabolic substances.

9838

Fat Metabolism of the Isolated Heart.

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It is the conclusion of Cruickshank[1] that the isolated heart derives its energy at least in part from the combustion of fat. This deduction is based on the negative evidence that combustion of other fuels will not account for the total metabolism. According to Visscher and Mulder[2] the total oxygen consumption of the heart-lung preparation cannot be accounted for on the basis of the carbohydrate loss from the tissue and blood in that system. They found that in some cases as much as 80% of the total metabolism is non-carbohydrate, presumably fat. Previous studies on fat burning by striated muscle are conflicting. Palazzolo[3] reported a decrease, while Winfield[4] found no change in the fat content of muscle as a result of activity.

In order to elucidate this question further measurements of the total fat content of the mammalian ventricular muscle were made in two series of cases. For the first series hearts were obtained directly from anesthetized dogs, and for the second ventricular

1 Cruickshank, E. W. H., *Physiol. Rev.*, 1936, **16**, 597.

2 Visscher, Maurice B., and Mulder, Arthur G., *Am. J. Physiol.*, 1930, **94**, 630.

3 Palazzolo, Giovanni, *Archivio di Fisiologia*, 1912-13, **11**, 558.

4 Winfield, G., *J. Physiol.*, 1914-15, **49**, 171.

muscle was taken after periods of work in the heart-lung preparation. The two series were run concurrently and there was no selection of the animals for the two groups. The ventricular muscle was ground and 20 gm. samples taken for extraction and analysis for total fats by the Bloor[5] technique. The heart-lung preparations were made by the usual method and no additions of any material were made to the blood during the course of the experiments.

Analyses were made on 63 hearts, of which 25 were worked for periods of from one to 6 hours, the average time being 3 hours.

TABLE I.
Total Fat Content of Ventricular Muscle (Figures in % of Wet Weight).

Unworked			Worked	
3.64	3.31	4.22	2.58	3.95
3.27	3.00	4.07	4.66	3.35
2.37	3.72	3.99	1.91	2.69
3.31	2.99	2.72	2.32	3.95
3.24	2.93	1.92	2.63	3.56
3.19	3.19	4.05	4.07	3.02
3.17	4.16	2.99	3.00	2.23
3.32	3.74	3.83	2.96	3.75
2.89	3.63	3.13	2.76	3.46
3.33	1.72	2.63	2.59	3.74
4.10	2.36	3.76	2.82	4.16
4.21	3.45	5.17	2.84	3.57
3.74	2.55		2.95	
Mean		3.71	Mean	3.18
Standard Deviation		0.76	Standard Deviation	0.68
Probable Error of Mean		0.083	Probable Error of Mean	0.091

Difference between mean fat contents	0.53
Probable error of the difference	0.123

The observed data are shown in Table I. It will be seen that there is great overlapping of values in the two series, as would be expected from the normal variability in fat content. However, the means of the two series differ by 0.53%, while the probable error of this difference is 0.12%. The mean difference is seen to be 4.3 times its probable error and therefore has considerable significance. The absolute difference is 14.4% of the unworked muscle value.

In spite of the fact that the difference observed is statistically quite significant it is still impossible to state categorically that the difference represents fat burned. Unfortunately the heart becomes edematous in time in the heart-lung preparation. An addition of 14.4% of non-lipid containing fluid to the heart muscle would account for the whole change observed. The water content has been determined and this figure does not differ by more than 2% in the worked and unworked hearts. However, this is due partly to the

[5] Bloor, W. R., *J. Biol. Chem.*, 1926, **68**, 33.

fact that hemorrhages occur into the heart muscle, thus increasing its mass by the addition of whole blood or corpuscles. In this case the water content would not be expected to change greatly. Nevertheless if whole blood or corpuscles account for the increase in heart mass the error introduced in the fat analysis is minimized because the total fat content of the corpuscles is high. No way has been found to measure accurately the extent of the error which may be introduced by the occurrence of edema or interstitial hemorrhage. It seems unlikely that these factors could account for the whole difference observed. Even if it were responsible for a half of the observed difference there would remain a substantial probability that fat was also burned. The results are presented as an evidence that fat is utilized by the actively metabolizing heart muscle, recognizing that they do not constitute positive proof because of the possibility that a part of the decline in fat content may be only apparent, due to cardiac edema.

9839

Digitalis and Coronary Blood Flow.

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Considerable work has been done in an effort to evaluate the effect of digitalis on coronary blood flow. A wide variety of methods have been employed and the results on the whole have been contradictory. Gilbert and Fenn[1] have reviewed the pertinent literature which preceded their report. These workers, after an extensive series of acute experiments in which they studied the effect of a number of preparations of digitalis on the outflow from the coronary sinus of the dog by use of the Morawitz cannula, concluded that digitalis preparations may exert a vasoconstrictor action on the coronary arteries.

The use of the Morawitz cannula requires deep anesthesia, an open thorax and artificial respiration; consequently, the period of observation is necessarily relatively brief. For a number of years

[1] Gilbert, N. C., and Fenn, G. K., *Arch. Int. Med.*, 1932, **50**, 668.

we have been studying coronary blood flow in the trained animal.[2] It has been possible to measure the blood flow in one of the coronary arteries of the dog as often as desired, and in some experiments for as long as 2 weeks. Formerly we used the thermostromuhr method of Rein, but recently we have employed the method described by Baldes and Herrick,[3] which makes use of a direct current heater. We have, therefore, been able to digitalize animals by divided doses in a manner comparable to the clinical method and at the same time observe the effect on the coronary blood flow.

Seven dogs were prepared for these experiments. They were trained to lie quietly and subsequently the thermostromuhr unit was placed on the circumflex branch of the left coronary artery under general anesthesia and with sterile technic as described in a previous paper.[2] Three of the animals fulfilled the following requirements sufficiently well to permit their use in this study: 1. The body temperature and pulse rate of the dog must have remained within normal limits for at least 24 hours following the operation. 2. The coronary blood flow must have remained relatively constant for a like period. 3. Prior to injection of the drug the dog must have taken food without hesitation and behaved in all respects like a relatively healthy dog. Since the results of the experiments were comparable, the data on only one of the animals are given.

The coronary blood flow of a dog weighing 16 kg. was observed intermittently for a period of 9 days. Successive doses of digitalis (digiglusin, Lilly) were given intramuscularly which, according to clinical standards, should have been more than sufficient to digitalize the animal.

In addition to observing the blood flow, the blood pressure (Hamilton technic[4]) was observed and electrocardiograms (Fig. 1) were taken both before and after operation. Two days after operation digitalization was begun. One cat unit of digiglusin was given intramuscularly at each injection. The first dose was given at 10:00 a. m., the second at 4:57 p. m. The following day another injection was given at 11:15 a. m. The drug was not given on the fourth but the injections were resumed on the fifth postoperative day when an injection was given in the morning and another in the afternoon. On the sixth day following operation the sixth and last

[2] Essex, H. E., Herrick, J. F., Baldes, E. J., and Mann, F. C., *Am. J. Physiol.*, 1936, **117**, 271.

[3] Baldes, E. J., and Herrick, J. F., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 432.

[4] Hamilton, W. F., Brewer, George, and Brotman, Irving, *Am. J. Physiol.*, 1934, **107**, 427.

dose was given. The blood flow was measured daily, from immediately after the operation until a break occurred in the heating circuit of the unit on the tenth postoperative day.

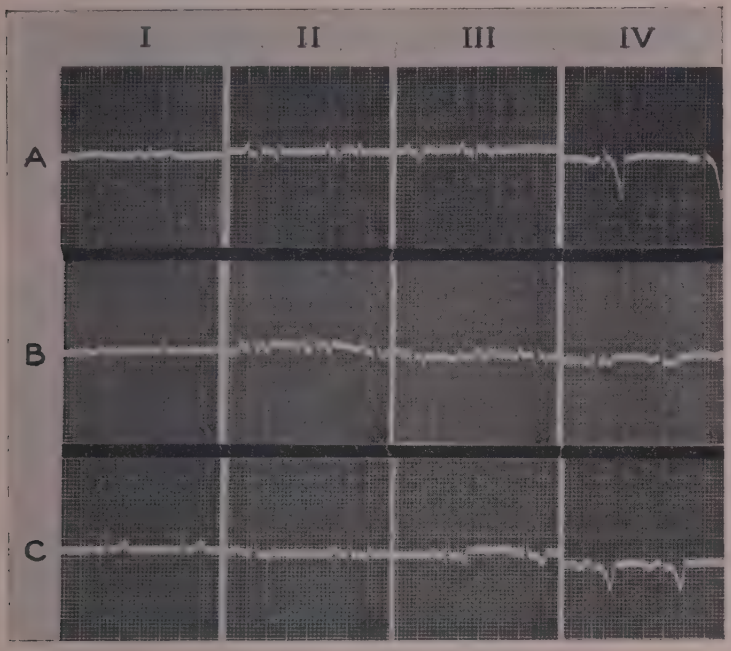

FIG. 1.

Electrocardiograms taken: A, 8 days before operation, and B, one day, and C, 7 days, following operation.

Judging by all the criteria employed, which consisted of observations of the electrocardiograms, blood pressure and rectal temperature, the condition of the dog was as close an approximation to normal as we have seen in the large series of animals that we have used in our experiments on coronary blood flow. As another indication of the splendid condition of the animal the unit was removed on the fourteenth postoperative day and an uneventful recovery followed.

On the basis of this series of observations it can be stated that digitalization with the form of the drug employed by us did not significantly change the blood flow in the circumflex branch of the left coronary artery of the dog (Fig. 2). An investigation of the effect of certain digitalis glucosides on coronary flow was recently

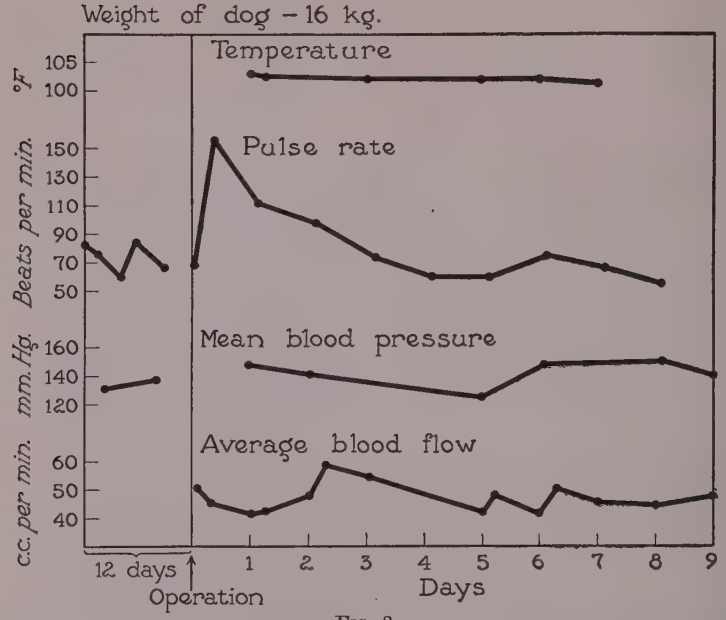

FIG. 2.

Summary of data on rectal temperature, pulse rate, blood pressure and blood flow. Six cat units of digiglusin were given from the second to the sixth post-operative day.

completed in collaboration with Dr. Maurice B. Visscher. The results, which are comparable to those reported here, will appear elsewhere.

9840

Failure of Sulfanilamide to Prevent Hemolysis, Fibrinolysis, and Production of Erythrogenic Toxin by Hemolytic Streptococci *in vitro*.

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The mode of action of sulfanilamide in hemolytic-streptococcal infections is still unexplained. The bacteriostatic effect, which can

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sometimes be demonstrated *in vitro*,[1] might conceivably be but one expression of a profound temporary change in the organism's activities. *In vivo* experiments have suggested that sulfanilamide renders streptococci more readily phagocytized.[2] Thus one might suspect an effect of sulfanilamide on the toxic substances produced by streptococci, and look either for inactivation of these agents or inhibition of their production.

From the finding that bone-marrow cultures preserve their morphology in the presence of copious streptococcal growth if the medium contains minute amounts of sulfanilamide, Osgood[3] suggests that the drug inactivates hemolysin and perhaps other toxic products as well. The trivial antihemolytic effect that he observed with sulfanilamide in blood-agar plates was attributed to impaired diffusion.

In view of Osgood's stimulating hypothesis, it was decided to investigate the action of sulfanilamide on hemolysis and fibrinolysis by broth cultures of hemolytic streptococci. Production of erythrogenic toxin and possible neutralization of this toxin were also studied.

The technical procedures were simple. Sulfanilamide was added to culture medium, hemolytic or fibrinolytic systems, and toxin-dilutions, from a stock solution of 200 mg. % in saline. The solution was sterilized by filtration, without appreciable loss of the drug. The solution was kept in the refrigerator, and, although no precipitation was observed, was warmed before use. The observations recorded were controlled with appropriate dilutions of sulfanilamide, which were found to be devoid of hemolytic, fibrinolytic, or erythrogenic effect.

The 4 streptococcal strains used were, "McGrew," freshly isolated from a child's mastoid, "ML-1A," an old septic-sore-throat strain from the National Institute of Health, the mouse-virulent "Todd," from Dr. H. M. Powell of Eli Lilly Co., and the scarlatinal N. Y. 5.

Concentrations of sulfanilamide of 15 to 40 mg. %, added along with rabbit blood to sugar-free broth, produced a slight delay in hemolysis when the tubes were inoculated. Such tubes showed little hemolysis 2 hours after inoculation, when there was marked hemolysis in the sulfanilamide-free controls. However, at 7 hours or later little difference could be noted. It was interesting that despite gross hemolysis, both sulfanilamide- and control-tubes

1 Long, P. H., and Bliss, E. A., *J. A. M. A.*, 1937, **108**, 32.
2 Gay, F. P., and Clarke, A., *J. Exp. Med.*, 1937, **66**, 535.
3 Osgood, E. E., *J. A. M. A.*, 1938, **110**, 319.

showed a few intact red cells in the sediment. The broth was not hemolytic.

Quite similar results were obtained when 16-hour cultures in 0.05% glucose broth were added to suspensions of washed human erythrocytes. After one hour in the waterbath there would be marked hemolysis by the control culture, and little by that grown in sulfanilamide-broth; but during the third hour the latter would rapidly catch up. Sulfanilamide 50 mg. % in the hemolytic system produced a similar effect in some instances, and none at all in others. Using 18-hour filtrates, Dr. Paul Hageman, to whom I am indebted for many unpublished data,[4] was unable to detect any antihemolytic effect of sulfanilamide.

Because turbidity appeared more slowly in sulfanilamide-media, it seemed likely that the slight early inhibition of hemolysis was due to growth-lag. DeKruif and Ireland[5] found that free hemolysin appeared in the medium only during very rapid growth, and was rapidly inactivated as growth slackened. Since sulfanilamide frequently flattens the growth-curve of *Str. hemolyticus,* the trivial effect on hemolysis is perhaps a little surprising. Living marrow-cells, if at all able to acquire tolerance for hemolysin, might detect slight hemolysis-inhibiting effects not readily demonstrable with peripheral erythrocytes. This is a possible explanation of the differences between these results and Osgood's. These findings are in accord with those of Swift, *et al.,*[6] which indicated that sulfanilamide did not inhibit the production of antigenic hemolysin in infected patients.

Fibrinolytic tests were done with the technic of Tillett and Garner.[7] Cultures were grown for 15 hours in 0.05% glucose-broth with and without 20 mg. % sulfanilamide. Each fibrinolysis-tube contained 0.2 cc. oxalated plasma, 0.5 cc. culture, and either 0.8 cc. saline or 0.4 cc. each of saline and stock sulfanilamide-solution. Coagulation was induced with 0.3 cc. of 0.25% calcium-chloride solution. No antifibrinolytic effect of sulfanilamide was noted. The results of a typical experiment are shown in Table I.

The remaining observations were on scarlatinal toxin. The broth used in this laboratory for the production of scarlatinal toxin is made with 0.1% glucose and 1% Parke-Davis "Bacteriological" peptone, in a veal-infusion base, and is autoclaved for 30 minutes to caramelize the glucose. No blood is added. With N. Y. 5 this

[4] Hageman, P. O., personal communication.

[5] DeKruif, P., and Ireland, P. M., *J. Inf. Dis.*, 1920, **26**, 285.

[6] Swift, H. F., Moen, J. K., and Hirst, G. K., *J. A. M. A.*, 1938, **110**, 426.

[7] Tillet, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

TABLE I.

Tube	Sulfanilamide solution, cc.	Saline, cc.	Culture	Time of lysis, min.
1	0.0	0.8	ML-1A, pl. broth	20
2	0.0	0.8	" "	15
3	0.0	0.8	" "	30
4	0.0	0.8	" sulf. broth	20
5	0.4	0.4	" pl. broth	15
6	0.0	0.8	Todd, "	60
7	0.0	0.8	" "	75
8	0.0	0.8	" "	90
9	0.4	0.4	" "	75
10	0.0	0.8	McGrew, "	65
11	0.0	0.8	" "	40
12	0.0	0.8	" "	40
13	0.0	0.8	" sulf. broth	70
14	0.4	0.4	" pl. broth	40
15	0.4	0.4	" "	45

pl. broth—without sulfanilamide.
sulf. broth—with 20 mg. % sulfanilamide.
sulfanilamide solution—200 mg. % sulfanilamide in saline.

broth regularly gives a titer of 50,000 STD/cc. A flask with 100 cc. broth and 25 cc. stock sulfanilamide-solution was inoculated with N. Y. 5; the 5-day filtrate was quite as potent as a toxin made in the same lot of broth without sulfanilamide. Children at the Shriners' Hospital were tested with 0.1 cc. of 1:500 and 1:5000 dilutions. Complete parallelism between the 2 toxins was observed in the 10 subjects with positive reactions to the higher dilution and in the 8 additional ones who reacted only to the lower.

For neutralization-tests the plain-broth toxin was diluted in saline with and without sulfanilamide. The tubes were then incubated at 37°C. for 30 minutes, and parallel skin-tests were done with 0.1 cc. amounts. Known positive reactors to toxin were used. Reactions to 1:1000 toxin in 10 mg. % sulfanilamide were studied in 15 subjects, and reactions to 1:5000 toxin in 200 mg. % sulfanilamide in 2 others. All of these reactions were indistinguishable from the controls. Dr. Hageman has found no neutralization of skin-toxin by 0.8% sulfanilamide. These findings are in accord with the clinical impression at this hospital that sulfanilamide is without effect on the purely toxic phase of scarlet fever.

Summary. Sulfanilamide produced a slight delay of hemolysis in blood-broth cultures of hemolytic streptococci. This effect was probably attributable to modification of the growth-curve. When used in concentration equal to or greater than that induced in the body-fluids therapeutically, sulfanilamide was without apparent effect upon fibrinolysis or formation of erythrogenic toxin *in vitro*, and was unable to inactivate small amounts of toxin.

9841 P

Effects of Morphine on the Motility of the Human Ileum.

ARMAND C. FORSTER. (Introduced by A. B. Hertzman.)

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The opportunities for direct study of intestinal peristalsis in the living human are necessarily limited. The usual methods of investigation have consisted of radiographic procedures or of the introduction of various mechanisms into the bowel, the very presence of which in the bowel may create abnormal reactions. Advantage was taken of a case of intestinal prolapse or bowel externalization (involving lower portion of the ileum) in an otherwise healthy male to record daily the activity of circular and longitudinal muscle. The loop of bowel was approximately 12 inches in length with an artificial anus at its summit so that the lower limb of the loop was constantly empty.

The method of recording described by Alvarez[1] was adapted in the following manner: Two Wiggers myocardiographs were fastened to permanent non-absorbable sutures loosely encircling a common mass of circular or longitudinal muscle. Lines joining points of attachment of the myographs to either circular or longitudinal muscle crossed each other at their mid-points. Attachment was made to the loop of bowel being traversed by intestinal contents. The apparatus was so arranged that it could be set up in working order without altering the position of the patient and might be used with the patient in a lying or semi-sitting position. While conducting the experiments, the bowel was kept covered with normal saline, and kept warm by a 75 Watt drop light. A thin rubber dam was placed over all to prevent evaporation.

The following precautions were observed in all experiments: The patient was prepared in no way for the experiment with the exception that any medication he might have been taking was discontinued the night before. To obviate reactions to the mechanical stimulus, tracings were not taken until at least 20 minutes after the hook-up. No disturbance of any kind was allowed, either to the patient or to the bowel while recording. The experiment was usually discontinued when the patient began to complain of fatigue.

Continuous tracings of circular and longitudinal muscle activity were thus taken in 25 experiments before, during, and after the

[1] Alvarez, W. E., *Mechanics of the Digestive Tract*, 2nd Ed., 1928.

administration of pharmacopeal and clinical doses of a drug. The observance of drug effects was continued for approximately an hour and a half. The effects of recorded activity were also visually observed with regard to expulsion of fecal contents from the visible anus thereby allowing intestinal activity to be classified as being propulsive or non-propulsive in nature.

Morphine sulphate was given intramuscularly in ⅛ grain doses and continuous tracings were taken in the manner described. There were 10 separate administrations of morphine.

The number of peristaltic waves (propulsive activity) and mixing waves (non-propulsive activity) during the preinjection period serves as a satisfactory basis for comparison of pharmacodynamic effects on intestinal muscle.

A complete suppression of the peristaltic wave with an increase in the frequency of the mixing wave followed ⅛ grain of morphine in all trials. The effects first appear 2 to 4 minutes after administration. Circular and longitudinal muscle tone was increased in half the trials. ¼ grain of morphine decreased the frequency and amplitude of contraction of the mixing wave, and increased the tone of both muscle coats in all experiments. Depression of propulsive and non-propulsive activity was still more complete when a total of ⅜ grain of morphine had been given. Increases in tone did not progress beyond that following ⅛ or ¼ grain of morphine.

9842 P

Persistence of Sylvatic Plague.

K. F. MEYER AND B. EDDIE.

From George Williams Hooper Foundation, University of California, and the California State Department of Public Health, San Francisco.

In June, 1916, sylvatic plague was first demonstrated in San Mateo County by gross anatomical examinations of squirrels *(Citellus beecheyi)*. During the next 4 years infected rodents were again discovered by dissection. Between 1921 and 1931 no surveys were conducted. In 1932 to 1935 annually, a few hundred squirrels and rats were shot and autopsied; no gross anatomical lesions suggestive of plague were observed. During August and September of 1936, a total of 863 beecheyi squirrels were again examined with negative results. The fleas, which had been killed with chloroform, were re-

moved from the animals and collected in physiological salt solution. A total of 18,729 fleas were thus assembled in 355 pools, which were stored at 3°C. for approximately 10 to 11 months since suitable facilities to examine them were not available. In 1937 the insects collected from the squirrels of the same area were pooled in 155 lots, carefully washed with salt solution, ground in a mortar and then injected subcutaneously into guinea pigs. Of the 155 pools so tested 6 produced fatal plague infections within 6 to 8 days. Two of the locations in the County where infected fleas were found in 1936 were recognized as the same colony or series of burrows proven to harbor diseased squirrels in the summer of 1916.

These and similar observations indicate that sylvatic plague persists probably indefinitely in an area once invaded and that the gross anatomical examinations fail to detect rodent infections. Squirrels, just as rats, may harbor *P. pestis* without visible lesions. The biology of these latent infections is the subject of further investigations. Although the viability of *P. pestis* in stock cultures for many years is well known, the fact that the bacteria remained alive in the dead fleas soiled with a variety of microörganisms *(B. coli, B. proteus,* Cocci, etc.) held at ice box temperature is indeed noteworthy.

9843 P

Antiviral Substances to the Virus of Encephalitis (St. Louis Type) in Serums Collected in California.

B. F. HOWITT. (Introduced by K. F. Meyer.)

From the George Williams Hooper Foundation, University of California Medical Center, San Francisco, and the California State Department of Health.

During the summer of 1937 reports were obtained of an outbreak of acute encephalitis in the San Joaquin Valley region in California. A number of fatal cases were recorded and while brain material was received from 3 of these, no virus was recovered. Monkeys and mice were inoculated but all remained negative, even after repeated serial passages in the mice. Nasopharyngeal washings were also taken from 6 patients still hospitalized but no virus was recovered. Since the histopathology of the human material was typical of an acute encephalitis, serum was obtained through the courtesy of the Health Departments of both Fresno and Tulare

counties from the available cases of 1937 and from 2 diagnosed in 1936.

Serums from 29 people were tested for neutralizing ability against the viruses of lymphocytic choriomeningitis (l.c.m.) and the St. Louis type of encephalitis,* respectively. The blood was taken at different lengths of time, 15 days to 13 months, after the onset of the disease, the majority being obtained from 2 to 3½ months after the onset. The patients varied in age from 3 to 66 years, but the largest group was from 17 years upward. There were 10 under 16 and 19 over that age. All except 3 had recovered without particular aftereffects. The 3 exceptions showed cerebral symptoms and their serums failed to neutralize the St. Louis virus.

Blood was also obtained from 6 doctors and nurses who had been in contact with the patients during the outbreak but who had not contracted the disease. Two of the nurses reported having had poliomyelitis, one in Fresno in 1935 and the other in Los Angeles in 1934. Serums from a group of 9 nurses and students connected with the University of California were also tested.

The neutralization tests were performed as follows: A 10% mouse brain suspension was diluted in a mixture of equal parts of hormone broth and sterile distilled water to a 1-100 and occasionally 1-250 dilution, respectively, for the l.c.m. virus and to 1-10,000 and 1-50,000, respectively, for the other strain. Equal parts of each serum and of each dilution of virus were mixed, placed at 37°C. for one hour and then kept overnight in the icebox before injection of 0.03 cc. intracerebrally into mice. Both positive and negative serums were always included in each series.

It was found that no serums neutralized the l.c.m. virus, but that of the 29 from encephalitic patients, 16 or 55.1% gave positive neutralization against the St. Louis virus and 13 or 44.9% were negative. According to districts: of the 12 serums from Fresno 4 were positive, including one from a case diagnosed in 1936, while from Tulare 12 of the 17 serums gave positive neutralization. In the latter region the diagnosis of "polio-encephalitis" was largely made for these cases from whom the serums were obtained, headache and general malaise being usual symptoms. No residual paralysis was noted for this group.

Of the 6 serums from contacts only one neutralized the St. Louis virus and that was from the nurse that had been diagnosed as having had poliomyelitis in 1935 with some paralysis from which a good

* The former virus was kindly sent by Dr. C. Armstrong of the National Institute of Health and the latter by Dr. L. T. Webster of the Rockefeller Institute.

recovery was made. The serum was removed about 2 years after the onset. All of the non-contact serums were negative.

McCordock, Smith and Moore[1] reported another outbreak of encephalitis in the St. Louis region in 1937 while Brodie[2] describes having tested the l.c.m. and the St. Louis viruses, respectively, against serums from patients in New York who had recovered from non-paralytic poliomyelitis in 1935. All failed to neutralize either type of virus. It might seem, therefore, from the results recorded here that the virus of acute summer encephalitis has been traveling west rather than east after the 1933 epidemic.

Summary. Of 29 serums from patients diagnosed as having had acute encephalitis or a polio-encephalitis, respectively, in one region of California none neutralized the virus of lymphocytic choriomeningitis, while 16 or 55.1% gave a positive test with the St. Louis type of encephalitic virus.

Appreciation is extended to Dr. E. R. Zumwalt and his staff at Tulare, to Dr. H. M. Ginsburg and the group at Fresno for their kind coöperation and the many courtesies offered and to Dr. C. Hawley for the collection of many of the serums.

9844 P

Synthesis of the Antihemorrhagic Vitamin by Bacteria.

H. J. ALMQUIST, C. F. PENTLER AND E. MECCHI. (Introduced by K. F. Meyer.)

From the Division of Poultry Husbandry, College of Agriculture, and the George Williams Hooper Foundation, University of California, San Francisco.

Fish meal and rice bran which have been exposed to the action of microörganisms contain the antihemorrhagic vitamin-K.[1] This factor has also been demonstrated in the feces of chicks on a K-free diet.[2] The present work is a preliminary investigation of the production of the vitamin by bacterial synthesis.

It was again found that the putrefaction of ether-extracted, K-free fish meal was accompanied by the formation of appreciable

[1] McCordock, H. A., Smith, M. G., and Moore, E., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 288.

[2] Brodie, M., *J. Inf. Dis.*, 1937, **61**, 139.

[1] Almquist, H. J., and Stokstad, E. L. R., *J. Biol. Chem.*, 1935, **111**, 105.

[2] Almquist, H. J., and Stokstad, E. L. R., *J. Nutrition*, 1936, **12**, 329.

amounts of a fat-soluble, antihemorrhagic substance. Several different types of bacteria were present in the putrefied material. An organism producing a similar putrefaction was isolated in pure culture* and inoculated to wet, sterile, ether-extracted fish meal. As a control, a similarly prepared uninoculated fish meal was used. Following 10 days' incubation, when a strongly putrid odor had been developed in the inoculated sample, the material was extracted while still moist by shaking with ether. The ethereal extract, the extracted residue, and the sterile control fish-meal sample were each added to different portions of K-free chick-ration[3] and each portion was fed to day-old chicks for a period of one week. The ethereal extract of putrid fish meal and the extracted residue each contained sufficient antihemorrhagic factor to maintain an approximately normal blood-clotting time of chicks.[3] The sterile fish-meal control gave no evidence of activity.

The fish-meal organism was then inoculated to beef broth, fish-meal broth, proteose-peptone broth, gelatin, and nutrient agar. Washed bacteria from broth and from nutrient agar were found to be rich sources of the antihemorrhagic factor. Liquid media from which the bacteria had been removed by filtration, and saline used in washing the bacteria were negative. Sterile media used as controls were all negative.

Several known species of bacteria were grown in small amounts on

TABLE I.
Antihemorrhagic Vitamin Assays.

Supplement to basal diet	Level per kg. of diet, gm.	No. of chicks	Aver. blood clotting time after 1 wk., min.
None (negative control)	—	5	>30
Alfalfa extract (positive control) equivalent to dry weight of	5	5	3.5
Fish meal organism, undried	5	4	3.9
" " " dried	0.63	5	3.1
Bacillus mycoides	—	5	3.1
Sarcina lutea	—	5	5.0
Bacillus subtilis	—	5	3.2
Chromobacterium prodigiosus	—	5	6.2
Staphylococcus aureus	—	5	3.9
Pseudomonas aeruginosa	—	5	>30
Bacterium coli	—	5	4.1
Bacillus cereus, dried	1.0	5	4.5
Sarcina lutea, dried	1.0	5	3.8
Mycobacterium tuberculosis (human) (an old, bouillon preparation)	2.0	5	4.3

* Closely resembles *Bacillus cereus*. Referred to in Table I as the "Fish-Meal Organism."

[3] Almquist, H. J., and Stokstad, E. L. R., *J. Nutrition*, 1937, **14**, 325.

nutrient agar, suspended in saline, washed repeatedly, autoclaved 40 minutes at 125°C., and added to the basal diet for assay as described elsewhere.[3]

The results given in Table I demonstrate that an antihemorrhagic factor is present in certain species of bacteria. It is evident that this factor is a product of the bacterial metabolism.

Dried bacteria of some species show from 5 to 8 times the antihemorrhagic activity of dried alfalfa. In contrast, preparations of *Pseudomonas aeruginosa* had no demonstrable activity, and other species of bacteria may also prove to be poor sources. Yeast is another microörganism which contains little or none of the vitamin.

The antihemorrhagic factor from bacteria is also extractable by fat-solvents, but its further similarity to the vitamin-K from alfalfa[4] remains to be established. The distribution of this factor, the manner of its production, and its significance in bacterial metabolism are matters for further investigation.

9845 P

Effect of Pregnancy on the Growth of Rat Sarcoma.*

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In previous publications we reviewed the influence of pregnancy on tumor growth (Emge,[1] Emge and Wulff[2]) and showed that in transplantable mammary rat adenofibroma, fibroma, and sarcoma developed in our laboratory the growth rate either was not influenced or was at times only slightly retarded. From a more recent study[3] we learned that rapidly repeated pregnancy does not affect the adenomatous component of our transplantable mammary rat adenofibroma 5-B 1 beyond that expected in a single pregnancy,

4 Almquist, H. J., *J. Biol. Chem.*, 1937, **117**, 517; 1937, **120**, 635.

* Aided by grants from the Rockefeller Fluid Research Fund of Stanford University School of Medicine, and from the Scientific Research Committee of the American Medical Association.

1 Emge, L. A., *Am. J. Obst. and Gynec.*, 1934, **28**, 682.

2 Emge, L. A., and Wulff, L. M. R., *Western J. Surg. Obst. Gynec.*, 1934, **42**, 45.

3 Emge, L. A., and Murphy, K. M., PROC. SOC. EXP. BIOL. AND MED., 1938, **37**, 620.

and that wide variations in the daily weight gain of these tumors during pregnancy are not unlike those occurring in control animals.

In order to obtain further information on the influence of pregnancy on tumor types developed by us we undertook a study of the behavior of certain strains of sarcoma in relation to the number of days exposed to pregnancy.

Of several hundred mature animals implanted with rat sarcoma E, developed by us from transplantable mammary adenofibroma, 31 became pregnant during the growth of the original implant and 48 during the growth of a recurrent tumor. The growth rate was studied in relation to the percentage of time that each animal was pregnant.

TABLE I.

	Cases	Mean daily rate of growth in gm./day
Original implants	31	0.95
First recurrences	48	1.29
	79	Diff. 0.34

Since there are approximately 25 chances in 100 that such a difference of ±0.34 gm./day would occur simply as a result of sampling error, it was considered proper to combine both categories into a single group.

Pearson's coefficient of correlation (r) is –0.1889,† suggesting an inverse correlation between the growth rate and the percentage of time pregnant. The negative correlation, however, may be due to the use of ratios. The standard error of r (on the assumption that its true value is zero) is $1/\sqrt{N-1} = 1/8.8318 = 0.1132$. Thus the relative deviate (x/σ) equals 1.6687.

The probability that an absolute value of r as great or greater than that obtained would arise from a sample of this size by chance alone is 0.095, or roughly, 1 in 10, which is statistically of no significance. Therefore, there is no reason to believe that the growth rate of this rat sarcoma is influenced by pregnancy.

In 2 additional experiments we studied the growth rate of implanted mammary rat sarcomas‡ E-2 and E-5 during pregnancy and during a similar period of recurrence after littering.

Implant with Sarcoma E-2. Eighteen pregnant animals and 9 female controls 90 to 103 days of age were implanted. In the preg-

† The calculations were made on ungrouped data.

‡ It is emphasized that the sarcoma strains used in these experiments are morphologically similar and derived from the same stem.

nant group tumors were removed on the day of littering (from the 17th to the 20th day of tumor growth). In the control group all tumors were removed 20 days after implant. Because of the relatively faster growth of the recurrent tumors in the puerperal group (although this difference was later found to be of no significance), they were removed 13 to 16 days after littering. The experimental results with the calculated value of P§ are shown in Table II.

TABLE II.

	Cases	Av. gain in gm./day: Original Implant	Av. gain in gm./day: Recurrent Tumor	Diff.	P§
Pregnant	18	1.79	2.06	.27	.245
Control	9	2.44	2.58	.14	.788
Diff.		.65	.52		
P		<.01	.302		

Implant with Sarcoma E-5. Six pregnant animals and 5 female controls 107 to 120 days of age were implanted. Tumors were removed immediately after littering and on the 21st day following implantation, respectively. Recurrent tumors were removed at the end of a period equalling the growth period of the original tumor. The experimental results and P values are shown in Table III.

TABLE III.

	Cases	Av. gain in gm./day: Original Implant	Av. gain in gm./day: Recurrent Tumor	Diff.	P
Pregnant	6	.95	1.50	.55	.113
Control	5	1.69	1.69	0	1.000
Diff.		.74	.19		
P		.029	.731		

The results are comparable with those of the previous experiment.

Conclusions. 1. In the 3 types of sarcomas, E, E-2, and E-5, studied here, no significant difference was observed between the growth rate of the original implant and the recurrent tumor. This applies to controls as well as to pregnant animals. 2. The slight correlation existing between the growth rate of sarcoma E and the percentage of time the tumor was acted upon by pregnancy is not

§ P = probability of a difference as great or greater than that obtained occurring by chance alone. By accepted standards, values of .05 or less (*i. e.*, 5 per hundred or under) are considered significant.

significant. 3. The original implants of sarcomas E-2 and E-5, when implanted into pregnant animals, showed a significantly lower growth rate than those implanted into controls. No significant differences were observed, however, in the recurrent tumors.

9846 P

Phosphorus Fractions in Human Heart Muscle.

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In pursuance of our studies of the myocardial chemical changes that accompany congestive heart failure, we have attempted to determine the various phosphorus compounds in the left ventricles of hearts obtained at autopsy. These have been divided into two groups, those with and those without myocardial failure. It is well known that many hearts that have failed give no hint of their lack of functional capacity when examined histologically and previous studies[1] indicate that changes in the chemical compounds participating in the energy exchanges of muscular contraction accompany, and may play a causative rôle in the precipitation of congestive failure.

The total myocardial phosphorus is composed of that present in those compounds which are soluble in 5% trichloracetic acid, namely, phosphocreatine, hexose phosphates, nucleotid phosphoric acid, and inorganic or orthophosphate, plus that residual portion which is not acid-soluble. The residual phosphorus has been identified with the phospholipid fraction by Sorg[2] and Wassermeyer.[3]

It has been previously shown that a decrease in total phosphorus accompanies myocardial failure.[4] The hypothesis has been advanced that the endurance of a muscle parallels its phospholipid content,[5] and in agreement with this idea, Kutchera-Aichenberger[6] reported phospholipid decreases in the heart in congestive failure and in ex-

1 Herrmann, G., and Decherd, G., *Trans. Assn. Am. Phys.*, 1936, **51**, 295.
2 Sorg, K., *Z. f. physiol. Chem.*, 1929, **182**, 97.
3 Wassermeyer, H., *Deut. Arch. klin. Med.*, 1934, **177**, 573.
4 Wilkins, W. E., and Cullen, G. E., *J. Clin. Invest.*, 1933, **12**, 1063.
5 Lehnartz, E., *Erg. Physiol.*, 1933, **35**, 874.
6 Kutchera-Aichenberger, H., *Wien. Arch. f. inn. Med.*, 1929, **18**, 209.

perimental myocardial injury by chloroform. However, Wassermeyer and Rohrbach[7] found no changes in the lipoids of the heart in experimental valvular lesions, with or without hypertrophy, in myocarditis produced by caffeine and adrenaline, or in beri-beri. Furthermore, Ludewig and Chanutin[8] could detect no change in the lipoid phosphorus of hearts hypertrophied secondarily to hypertension in partially nephrectomized rats.

It is to be emphasized that the abrasive used in grinding the muscle for extraction must be devoid of any adsorptive properties, else the determined acid-soluble phosphorus will be too low and, by difference, the residual (or lipoid) phosphorus, too high. Detection of this source of error in our preliminary determinations[9] necessitated this study now reported.

Methods. Duplicate weighed portions of heart muscle, obtained within 48 hours after death, were taken for (a) digestion with sulphuric acid and superoxol for estimation of the total phosphorus; (b) ground with washed sea sand, which was shown to adsorb none of the soluble phosphorus compounds, and extracted with a calculated volume of 5% trichloracetic acid, so that 20 cc. of extract represented 1 gm. of muscle, assuming the muscle to contain 80% water; portions of this extract were digested to determine the total acid-soluble phosphorus, and in addition were fractionated according to the technique of Pollack, *et al.*;[10] (c) dried at 105°C. to constant weight. Phosphorus determinations were done by the method of Fiske and Subarrow,[11] and all values are given in terms of 100 gm. fresh muscle, being corrected to a standard of 20% content of solids for purposes of comparison.

Results. We have confirmed in a larger series the drop in total phosphorus in the muscle of hearts that have failed, observed by Wilkins and Cullen. This decrease is due entirely to a decrease in the acid-soluble fraction. There is no change in the residual phosphorus. The mean of each series, expressed as milligrams of phosphorus per 100 gm. of fresh muscle, with the probable error of the mean, as well as the standard deviation, have been calculated and assembled in Table I; the lower line gives the difference between the means of the 2 large groups, namely, hearts with and without failure, and the probable error of this difference.

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7 Wassermeyer, H., and Rohrbach, A., *Arch. f. exp. Path. u. Pharm.*, 1932, **166**, 375.

8 Ludewig, S., and Chanutin, A., *J. Biol. Chem.*, 1936, **115**, 327.

9 Herrmann, G., and Decherd, G., unpublished data.

10 Pollack, H., Flock, E., and Bollman, J. L., *Am. J. Physiol.*, 1934, **110**, 105.

11 Fiske, C. H., and Subarrow, Y., *J. Biol. Chem.*, 1925, **66**, 375.

TABLE I.
Phosphorus Values in Human Heart Muscle.
Mg. per 100 gm. Fresh Muscle.

		Total P Mean ± PE	σ*	Acid-soluble P Mean ± PE	σ*	Residual P Mean ± PE	σ*
No Failure	36	180.3 ± 0.90	8.0	88.8 ± 0.99	8.4	91.0 ± 1.04	9.3
Failure	33	161.4 ± 1.15	9.8	72.3 ± 0.96	8.2	89.0 ± .91	7.7
Difference		18.9 ± 1.46		16.5 ± 1.38		2.0 ± 1.38	

*of the whole series.

The attempts to fractionate the acid-soluble fraction were unsuccessful. As might be anticipated, these labile compounds had hydrolyzed before we obtained the muscle; the estimation of the changes in this group must await the experimental production of heart failure. In a few instances, traces of soluble barium salts were obtained (phosphocreatine or hexosephosphate), but these were too small to measure. No pyrophosphate could be identified.[12] The orthophosphate was consistently 5 to 15 mg. % lower than the total acid-soluble phosphorus; this difference possibly is a measure of a portion of the nucleotid phosphorus which is either difficult to hydrolyze or is as yet unidentified.

Summary. The decrease in total myocardial phosphorus which accompanies heart failure is due to a decrease in the acid-soluble phosphorus compounds.

9847 P

Use of Mice for Testing Toxicity of Rabbit Therapeutic Antipneumococcic Serum.

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Since the newly developed *rabbit* therapeutic (unconcentrated) antipneumococcic sera, now being used with favorable clinical results,[1, 2, 3] even after processing of the raw serum by heating at 56°C.

12 Lohmann, K., *Biochem. Z.*, 1928, **202**, 466.

1 Horsfall, F. L., Jr., Goodner, K., and MacLeod, C. M., *Science*, 1937, **84**, 579.

2 Horsfall, F. L., Jr., Goodner, K., MacLeod, C. M., and Harris, A. H., 2nd, *J. Am. Med. Assn.*, 1937, **108**, 1463.

3 Horsfall, F. L., Jr., Goodner, K., and MacLeod, C. M., *N. Y. State J. Med.*, 1938, **38**, 246.

and absorption with kaolin,[4] have been found to cause chills in a considerable proportion of patients, the importance of testing each lot of rabbit antiserum for its chill-producing quality before injection into man has been emphasized.[3, 4] It is recommended that each of 3 *rabbits* be inoculated intravenously with 2.0 cc. of the processed rabbit antiserum and that their rectal temperatures be determined before, and one hour after, the injections. If the mean thermal elevation exceeds 1.3°F. it is said that the serum will produce chills in patients, whereas lots of serum "negative in rabbits" will not do so.[3, 4]

It occurred to us that a toxicity-test utilizing a different species of animal than that from which the antiserum is derived, and not dependent entirely upon slight changes in rectal temperatures, would probably prove more satisfactory, and since the necessary technic for intravenous injection was familiar,[5, 6] we have investigated the possibility of using *mice* as test-animals.

Five rabbits, 3 of which had been immunized to egg-white and 2 to typhoid bacilli, were bled aseptically; the toxicity of these sera, before and after processing by Goodner's method,[4] was tested by intravenous injections of mice and rabbits whose rectal temperatures were taken 15, 30, and 60 minutes after injection, and when indicated, at 90- and 120-minute intervals.

Preliminary trials showed that the raw rabbit serum from *each* of the 5 rabbits was equally toxic for mice and that 1.0 cc. was close to the minimal lethal dose when given *intravenously*.

TABLE I.
Toxicity of Raw and Processed* Rabbit Serum for Mice and Rabbits.

Test animals	No. inoculated	Inoculum: Rabbit serum	Inoculum: Dose, cc.	Symptoms	Aver. max. temp. change °F	Died
Rabbits	6	Raw	2.0	None	+0.2	0
"	14	Processed	2.0	"	+1.0	0
Mice	14	Raw	1.0	Severe, prolonged	>—4.4†	11
"	9	Processed	1.0	Slight and transient,	+1.2	0
"	8	"	1.5	or none	—2.5	0

*Sera processed as follows: (1) heated at exactly 56°C for 30 minutes, (2) mixed with sterile kaolin and refrigerated overnight, (3) centrifuged to remove kaolin, (4) heated again for one-half hour at 56°C.

†Ten animals only.

[4] Goodner, K., Horsfall, F. L., Jr., and Dubos, R. J., *J. Immunol.*, 1937, **33**, 279.

[5] Burdon, K. L., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 340.

[6] Burdon, K. L., *J. Lab. and Clin. Med.*, in press.

The results of our comparative tests of the same sera in mice and in rabbits are summarized in Table I.

These experiments demonstrated the great reactivity of mice toward the toxic qualities of rabbit serum, and indicated that toxicity of this serum may be regularly and easily demonstrated in mice in an unmistakable manner. The mice appeared to be definitely superior to rabbits as test animals since their reactions to toxic rabbit sera were more uniform and far more determinate.

Contrary to a general impression, no real difficulty was presented by the necessity of making intravenous injections in the test mice, for, with the use of a suitable holder,[6] these inoculations may be made rapidly and accurately by a single worker.

The fact that the elements in rabbit sera toxic for mice were removed by a method which Goodner has found effective in reducing chill reactions in man, makes it probable that a serum, so processed, causing no reaction in mice, would also be non-toxic for human beings.

Unfortunately we are unable to report at this time complete tests of this hypothesis because samples of the unconcentrated therapeutic antipneumococcic rabbit sera, now undergoing clinical tests by Horsfall, Goodner, and their collaborators, of known chill-producing potentialities in man, have not as yet been furnished us.

The usefulness of mice for toxicity tests is at present being investigated further with samples of the concentrated therapeutic antipneumococcic rabbit sera now being developed by the Lederle Laboratories.

Conclusion. The intravenous injection of mice may prove to be a more satisfactory procedure than the methods now in use for testing the potential toxicity of rabbit antisera intended for human therapy.

9848

Organs as a Source of Factors Capable of Eliciting the Shwartzman Phenomenon.*

WILLIAM ANTOPOL AND DAVID GLICK.

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In a previous investigation[1] it was shown that fractions prepared from tumor tissue were capable of producing the Shwartzman phenomenon. The present study was extended to include normal tissues, and it was found that in a number of cases the factors capable of eliciting the Shwartzman phenomenon were present in these tissues as well. Though a variety of organs were tested in this connection for provocative potency a more complete investigation was made of bovine pancreas and testis, and rabbit liver as sources of both preparatory and provocative factors.

The tissues employed were obtained from the operating room or from freshly killed animals. Bacteriological studies of the fresh animal organs were not always made because of the lack of facilities at the abbatoir. Whenever possible, cultures were made on blood agar plates, semi-solid agar, and anerobic sheep brain media. The organs which were cultured are indicated in the tables. A portion of the tissue was removed for histological examination and the remainder was treated immediately in the manner previously described.[1] It is obvious that no special precautions to maintain sterility were needed since every step in the preparation of the fractions was of such a nature that sterility was automatically maintained. The dried fractions thus obtained were dissolved in physiological saline at 100°, just prior to use and these solutions were tested against the extracts obtained from bacteria as previously described.[2]

It may be seen from the tables that fractions from bovine pancreas and testis, and rabbit liver contain both preparatory and provocative factors. Sheep pancreas and testis fractions were found to act similarly. An occasional preparation was found to be inactive regardless of the dose administered. The effects of preparations from a variety of other organs are apparent from Tables I and II. Heart preparations proved fatally toxic to the rabbits though at the time of death there was indication of the onset of positive reactions.

* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

1 Antopol, W., *J. Infect. Dis.*, 1937, **61**, 333.

2 Antopol, W., *J. Infect. Dis.*, 1937, **61**, 331.

TABLE I.

Various Preparations as Provocative Factors.

Source	Organ	Preparation used	Provocative dose (mg.)	Reactions in rabbits‡
	Skin preparatory dose 1 mg. *B. proteus* preparation.			
Cow	Liver†	A	50	3/0
Rabbit	" *	N	"	2/1
Cow	Muscle	A	"	1/2
"	Kidney*	A	"	2/1
"	Spleen†	A	"	0/2
Sheep	Brain†	B	"	2/0
Human	Placenta*	M	"	2/1
"	Uterus*	N	20	0/1
"	"	N	55	1/0
"	Breast*	O	12	1/0
Cow	" (lactating)*	L	20	0/1
"	" "	L	50	1/0
"	Pancreas	C	"	0/1
"	"	C	75	1/0
"	"	D	100	1/0
	Skin preparatory dose 20 mg. *B. typhosus* preparation.			
Cow	Pancreas	A	50	2/1
"	"	A	100	8/0
Bull	Testis	F	50	2/0
"	"	G	75	1/0

*Tissues bacteriologically sterile.

†Diphtheroids cultured aerobically, sterile anaerobically.
(All other tissues not cultured.)

‡The results are indicated according to the convention of Shwartzman and Morell.[6] The numerator denoting the number of positive and the denominator denoting the number of negative animals.

TABLE II.

Source	Organ	Preparation used	Skin preparatory dose (mg.)	Provocative dose extract of *B. proteus* (mg.)	Reactions in rabbits
Cow	Pancreas	E	50	.125	1/1
"	"	F	50	.125	1/0
"	"	G	50	.125	0/1
"	"	G	25	1.0	1/0
"	" *	H	50	.25	1/0
"	" *	H	95	.25	1/0
"	" *	I	50	.25	0/1
Bull	Testis	A	50	.125	0/1
"	"	B	50	.125	0/2
"	"	B	50	.500	0/1
"	"	C	50	.125	0/1
"	" *	D	50	.25	1/0
"	" †	E	75	1.0	1/0
Rabbit	Liver*	N	50	.25	1/2
"	" *	S	50	.25	1/0

*, † refer to Table I.

[6] Shwartzman, G., and Morell, S., *J. Exp. Med.*, 1938, **67**, 1.

The presence of Shwartzman factors in normal tissues is not entirely unexpected since vascular lesions have been described in various organs without preliminary preparation, after the intravenous introduction of potent bacterial filtrates prepared in the manner described by Shwartzman[3, 4, 5] or with fractions prepared from the bacteria themselves.[2]

The possible rôle of Forrsman antigen-antibody combination in the mechanism of these reactions has been excluded in this instance, since the cow, which was the source for most of the tissues, is devoid of this heterophile antigen. In addition, fractions obtained from sterile rabbit liver, of the same species as the test animal, also contained preparatory as well as provocative factors.

Since the diphtheroids were cultivated from the organs used as sources of the fractions which gave negative Shwartzman reactions in certain instances, and in other cases, fractions gave positive reactions even though the tissues from which they were prepared were culturally sterile, it is reasonable to assume that this organism played no significant part in the elicitation of the reaction.

An attempt is being made at the present time to determine the nature of the potent factors involved.

Summary. Fractions prepared from pancreas, testis, liver, kidney, brain, placenta, muscle, breast, and uterus were shown to possess provocative Shwartzman phenomenon factors, although the results were irregular in some cases. Similar fractions from spleen gave negative results. Only the fractions from the first 3 of these tissues were also tested for preparatory potency, and were found to elicit positive reactions in some of the rabbits.

We wish to express our appreciation to Dr. Gregory Shwartzman of The Mount Sinai Hospital, New York, for his suggestions and criticism.

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3 Shwartzman, G., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 560; *J. Exp. Med.*, 1928, **48**, 247.

4 Apitz, K., *Virchow's Arch. f. path. Anat.*, 1934, **293**, 1.

5 Gerber, I., *Arch. Path.*, 1936, **22**, 776.

9849 P

Relation of Drug Action to a Cholinergic Mechanism at Sympathetic Synapses.

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Current pharmacological concepts embrace the possibility that drugs acting at junctions, where acetylcholine plays an important though perhaps not essential rôle in transmission, do so by exerting their influence on some step in the cholinergic mechanism.

The consistency of such a view was examined by studying the effects on a sympathetic ganglion of drugs known to act at the parasympathetic neuro-effector junctions, since both are sites where cholinergic systems operate. Pertinent observations may be found in papers by Langley,[1] by Dale and Laidlaw,[2] etc.

Action potentials of the tonic impulses normally coursing in the superior cervical preganglionic trunk of the rabbit (anesthetized with nembutal) and of the response in the postganglionic fibers were recorded by a Matthews oscillograph after amplification. The waves were also spread out for visual observation by a rotating mirror (speed corresponding to 1.8 meters per second) and converted into sound by a loud speaker.

Pilocarpine, representing the parasympathomimetic stimulants, and atropine, the depressants, were injected into the common carotid artery, all branches except to the ganglion having been ligated and cut and the carotid sinus nerve severed. Thus the ganglionic circulation was retained intact while allowing an effective concentration at the synapse before diffusion into the general circulation. This made possible the observation of local effects uncomplicated by distant actions such as on the central nervous system directly, or indirectly through the remaining innervated carotid sinus responding to vascular changes.

The increase in postganglionic impulses (compare records A and B, C and D) illustrates the stimulant effect of small doses of pilocarpine. This increase, seen again in E, F, took place without any change in the corresponding preganglionic records G and H, and consequently must have been due to a truly peripheral action at the synapse. A larger dose has a paralytic effect.

Atropine caused a reduction in postganglionic response, records

[1] Langley, J. N., *J. Physiol.*, 1878, **1**, 339.

[2] Dale, H. H., and Laidlaw, P. P., *J. Physiol.*, 1912, **45**, 1.

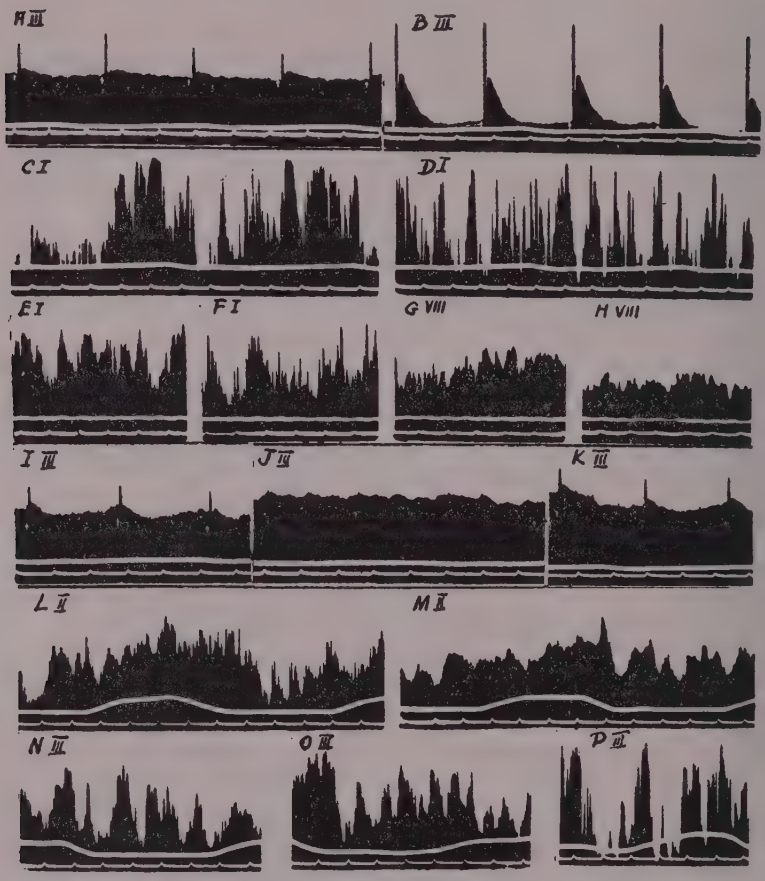

FIG. 1.

Postganglionic Potentials (except Preganglionic G, H).
Time in 1/5 sec. Inspiration = up, ↑ = submaximal fixed shocks 2/sec.
Sensitivity mm./10 uV. I = 3, II, = 4, III = 6, VIII = 20, before reduction.
Between A and B 2 mg./kg. (intraven.) Pilocarpine Nitrate.
" C and D, between E. G and F, H. 0.2 cc. 0.05% Pilocarpine Nitrate.
" I and J 2 mg./kg. (intrav.) Atropine Sulphate.
" L and M 0.2 cc. 1% Atropine Sulphate.
" N and O 0.2 cc. 1% Sol. Atropine : Pilocarpine = 1:10.
O to P, Pilocarpine action unmasked by Atropine destruction.

I, J, and L, M. Atropine is destroyed quickly in rabbits (K). It was, therefore, possible to repeat this several times in the same animal, a fact which was made use of later.

The specificity of the actions of pilocarpine and atropine is testified to by the fact that simultaneous injection of atropine and pilocarpine in the typical ratio of 1 to 10 resulted in an expected mutual

antagonism as described by Cushny[3] for the specific action of these 2 drugs on the salivary gland. Therefore, there is no change in impulses (N, O). Within 5 minutes the atropine is destroyed and the remaining pilocarpine causes an increase in impulses (O, P), which is cut down again by another dose of atropine.

To secure more readily interpretable and quantitative results a fixed stimulus, submaximal impulses (2 per second) from a thyraton stimulator, was used to activate the preganglionic trunk sectioned centrally. Stimulation was continuous from beginning to end of experiment. The rate was slow enough not to result unaided in facilitation at the ganglion, *i. e.*, before exhibiting drugs. Central effects being excluded by the section, the carotid branches were not disturbed and the drugs were injected intravenously. With this technique results identical with the above were more simply and strikingly brought out. Records A, B and I, J, K are examples illustrating the ganglionic effect of pilocarpine and of atropine.

It is concluded that so-called parasympathomimetic drugs act also on sympathetic synapses, responding to spontaneous or test stimuli, and might better be described as cholinotropic.

Cholinotropic is used in a descriptive sense to indicate that the action is exerted on cholinergic systems, but not necessarily through direct action with acetylcholine.

It is suggested that the relation between the action of certain drugs and the cholinergic mechanism at sympathetic synapses is significant and consistent with the view that drugs able to modify the cholinergic neuro-humoral process at this site may, amending predictions based on anatomical classifications, do so at all such sites with quantitative rather than qualitative differences. It is not, however, assumed that drugs may not also act at these sites independently of the neuro-humoral process.

[3] Cushny, A. R., *J. Physiol.*, 1903, **30**, 176.

9850 P

Gonadotropic Effect of Androgens upon the Immature Rat Ovary.

U. J. Salmon.* (Introduced by R. T. Frank.)

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Butenandt and Kudzus[1] have reported that androgenic substances administered to immature female rats cause premature opening of the vagina. The present study was undertaken to determine whether the androgens produce this effect directly upon the genital tract or indirectly through the ovary.

Eighteen rats (comprising 5 litters), varying in age from 27 to 30 days, were used in this study. Twelve were given a single injection of androgen in sesame oil; 6 controls were given an equal quantity of pure sesame oil. Seven of the animals were injected with testosterone propionate and 5 with androstenediol,† the dosage varying from 1 to 5 mg.

The time of the opening of the vagina was noted in each case. Laparotomies were performed and one ovary removed at intervals varying from 60 to 96 hours after the administration of the androgen. The animals were sacrificed at periods varying from 96 to 228 hours after the injection and the remaining ovary was then removed. At corresponding intervals, the ovaries were removed from the control animals. The ovaries were examined microscopically in serial sections.

Opening of the vagina occurred in all androgen-injected animals within 72 hours after the injection. Follicle stimulation was noted as early as 60 and 72 hours after the injection. Corpora lutea were found as early as 96 hours and as late as 192 hours after androgen administration. The ovaries of all the 12 injected animals exhibited some gonadotropic effect, either follicle stimulation, luteinization or both. The uteri in all these animals were markedly enlarged. In the 6 control rats, the vaginas remained closed and the ovaries were negative.

It appears from this study that testosterone propionate and androstenediol produce follicle growth and corpora lutea in the ovaries

* Hiram N. Vineberg Research Fellow in Gynecology.

1 Butenandt, A., and Kudzus, H., *Hoppe-Seyler's Z.*, 1935, **237**, 75.

† For the testosterone propionate and androstenediol used in this experimental study, I am indebted to Dr. Erwin Schwenk of the Schering Corporation, Bloomfield, N. J.

of immature rats. The premature opening of the vagina which occurs following the administration of androgens is apparently brought about through a gonadotropic effect exerted upon the ovary. Whether this gonadotropic effect is produced by direct action of the androgen upon the ovary or indirectly through stimulation of the hypophysis has not been determined. To ascertain this point, similar experiments with hypophysectomized animals would have to be performed. However, in view of the fact that Hohlweg and Chamono[2] have been able to produce corpora lutea in the ovaries of intact immature rats with an estrogen (estradiol benzoate) but not in hypophysectomized animals, it seems likely that the androgens act in a similar fashion, stimulating the hypophysis to secrete the follicle stimulating and luteinizing hormones.

9851

Effect of Androgens on Exophthalmos in Rabbits.

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In earlier papers[1, 2] we have reported (a) that exophthalmos developed more frequently in male rabbits (60 vs. 40%), (b) that this sex difference was independent of the exophthalmos-promoting effect of thyroid insufficiency, (c) that castration in the male greatly inhibited the development of exophthalmos, and caused gradual regression of an existing exophthalmos,[3] and (d) that cryptorchidism with complete degeneration of the germinal epithelium did not cause regression of exophthalmos in rabbits.[4]

From these observations it appeared highly probable that the interstitial cells of the testis were producing some hormone which, in association with reduced thyroid secretion, increased pituitary activity and perhaps other endocrine and mineral imbalances, was capable of maintaining an existing exophthalmos.

On the basis of these observations we have carried out experi-

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2 Hohlweg, W., and Chamono, A., *Klin. Wchnschr.*, 1937, **16**, 196.

1 Marine, D., Rosen, S. H., and Cipra, A., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 649.

2 Marine, D., Rosen, S. H., *Am. J. Med. Sci.*, 1934, **188**, 565.

3 Marine, D., Rosen, S. H., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 354.

4 Marine, D., Rosen, S. H., *Am. J. Physiol.*, 1938, **121**, 620.

ments on rabbits using testosterone propionate (Perandren) and androsterone, generously donated by Ciba Products, Inc., and dehydro-androsterone prepared in this laboratory. These hormones were dissolved in sesame oil and injected into the subcutaneous and intramuscular tissues of the abdominal wall. Eighteen experiments have been carried out in a group of 22 rabbits. The principal data of 11 of these experiments are given in Table I.

These experiments include rabbits with thyroid and gonads intact, with thyroids or gonads removed, with partial thyroidectomy and gonadectomy, and with partial thyroidectomy and cryptorchidism. The parenteral administration of large doses of androgens has produced or increased exophthalmos only in those rabbits (1) that had had exophthalmos and recovered (Exp. 1), or (2) that had latent exophthalmos (Exp. 6), or (3) that had definite exophthalmos at the time of the injections (Exp. 7). In no instance was exophthalmos produced by synthetic androgens in rabbits that never had had thyroid deficiency exophthalmos even though they had been subjected to thyroidectomy, thyroid feeding, cryptorchidism or gonadectomy. In experiments Nos. 1, 4 and 5, the same rabbits were used for testosterone propionate (70 mg. in 8 days), androsterone (150 mg. in 8 days) and dehydro-androsterone acetate (533 mg. in 4 days). The degree of exophthalmos produced was greatest with testosterone propionate and least with dehydro-androsterone acetate, despite the enormous differences in dosage used. The exophthalmic reactions obtained were qualitatively similar to the capon's comb, or rat seminal vesicle reactions to these hormones.

Experiment 3 shows the rapid counteractive or curative effect of desiccated thyroid on exophthalmos produced by testosterone propionate. This observation may be correlated with the antagonistic effect of thyroxine or desiccated thyroid on the gonads (depresses ovulation and spermatogenesis in rats and produces hen feathering in male birds). In those rabbits in which exophthalmos was produced or increased by androgens a suggestive increase was often noted about 6 hours after the first injection and was definite on the second day (24-36 hours). Another feature is the rapid regression of the exophthalmos after stopping the injections. This regression was evident in 3 days and sometimes complete in 7 days. The possibility that the sesame oil might have caused the exophthalmos was eliminated by using the oil in the same dose in 2 of our most sensitive rabbits (30 and 99). No rabbit with intact thyroid or in which the thyroidectomy appeared to be complete (no thyroid tissue found at surgical or postmortem examination) developed

TABLE I.

Exp. No.	Rabbit	Sex	Thyroidectomy	Gonadectomy	Cryptorchidism	Androgens injected: Total Amt. (mg.)	Androgens injected: Duration (days)	Degree of Exophthalmos: Before (days) 7	3	1	After (days) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
						Test. prop.																			
1	30	M	5-1		12-29	70	8	0			0	0	2	4	3	3	3	4	3	2	2	2	1	2	2
	99	M	3-24	11-27		70	8	0			0	2	3	3	4	1	4	4	3	3	1	1	1	0	1
	55	M			2-12	70	8	0			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
						Test. prop.																			
2	30	M	5-1		12-29	40	4	1	0	0	1	2	2	2	3	1	1	1	1	1	0	0	0	0	0
	99	M	3-24	11-27		40	4	1	0	0	0	1	1	3	3	2	1	1	0	0	0	0	0	0	0
						Test. prop.																			
3	30	M	5-1		12-29	160	16	0	0	0	1	2	2	3	2*	3	2	2	1†	0	1	1	2	2	1
	99	M	3-24	11-27		160	16	0	0	0	0	1	1	4	3	4	3	2	2	1	1	1	3	1	2
						Andros.																			
4	30	M	5-1		12-29	150	8		0	0	1	1	2	2		2	2	2	2	2	2	1	0	0	1
	99	M	3-24	11-27		160	9		0	0	1	1	1	2		1	3	1	3	2	2	1	0	0	1
						Dehydro. ac.																			
5	30	M	5-1		12-29	533	4	0		0	0	1	2	1	1		0	0							
	99	M	3-24	11-27		533	4	0		0	0	1	2	0	0		0	0							
						Test. prop.																			
6	87	F	6-10	8-6		50	10	0			0	1	1	1	1	1	2	2	3	4	4	3		4	1
	89	M	8-6	8-6		50	10	0			0	1	2	2	2	3	3	4	4	5	4	5		4	4
	94	F		8-6		40	8	0	0	0	0	0	0	0	1	0	0	0	0	0		0	0	0	0
	95	M		8-6		40	8	0	0	0	0	0	0	0	0	0	1	0	0	0		0	0	0	0
						Test. prop.																			
7	91	M	6-10	8-6		30	3	4	4	4	3	5	4	4	4	4	2	2	4	2	3	4	0	2	0
	92	M		8-6		30	3	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0
						Test. prop.																			
8	02	M				40	4		0		0	0	0	0	0	0	0								
	03	M				40	4		0		0	0	0	0	0	0	0								
						Test. prop.																			
9	02	M	11-9		11-9	50	5		0	0	0	0	0	0	0	0	0	0							
	03	M	11-9		11-9	50	5		0	0	0	0	0	0	0	0	0	0							
						Test. prop.																			
10	55	M	8-11		2-12	75	8	0	0		0	0	0	0	0	0	0	0							
	94	F		8-6		55	8	0	0		0	0	0	0	0	0	0	0							
	95	M		8-6		55	8				0	0	0	0	0	0	0	0							
						Sesame Oil																			
11	30	M	5-1		12-29	10 cc.	5	0		0	0	0	0	0	0	0									
	99	M	3-24	11-27		10 cc.	5	0		0	0	0	0	0	0	0									

*First dose desiccated thyroid.

†Last dose desiccated thyroid—total 500 mg.

exophthalmos following the injection of androgens. We have no explanation as to why some rabbits failed to develop exophthalmos. Similar failures, however, occur in guinea pigs with pituitary extracts, as well as in rabbits following partial thyroidectomy. These failures indicate that other unknown factors must be favorable in order to obtain exophthalmos. There is some evidence that one of these factors is a disturbance of mineral metabolism, especially the Ca-P ratio. Four female rabbits received testosterone propionate. In only one—an ovariectomized rabbit with latent exophthalmos—did exophthalmos develop. The two with intact ovaries developed intense oestrus as determined by vaginal and vulval reactions and sexual receptivity.

Summary. The data here reported indicate that thyroid insufficiency associated with increased pituitary and androgenic activity (active or passive) are important factors favoring the development of exophthalmos in rabbits. The androgenic factor is believed to play a rôle in the percentile increase in the incidence of post-thyroidectomy exophthalmos in Graves' disease in males.

9852

Sugar Alcohols XIII. Primulatol and Glycogen Storage in the Rat.

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Roe and Hudson[1] demonstrated that β d-mannoheptulose is physiologically available to the rabbit which has a high tolerance for this heptose. In the studies of the fate of the sugar alcohols and their anhydrides in the animal body in which mannitol[2] was shown to be a precursor of glycogen the possible utilization of the corresponding sugar alcohol, β d-mannoheptitol became of interest. The β d-mannoheptitol was extracted from *Primula officinalis* by the method of Bougoult and Allard.[3] The compound melted uncorrected at 153°.

Rats were fasted for 51 hours. After this period the control

1 Roe and Hudson, *J. Biol. Chem.*, 1936, **112**, 443.

2 Carr, Musser, Schmidt, and Krantz, *J. Biol. Chem.*, 1933, **102**, 721.

3 Bougoult and Allard, *Compt. rend.*, 1902, **135**, 796.

animals were fed a diet of cacao butter and the experimental animals a mixture of 1 part primulatol and 2 parts cacao butter. After 72 hours all the animals were killed under amytal anesthesia and their livers were immediately extirpated. The glycogen was determined by Good's[4] modification of Pflüger's[5] method and the glucose determined by the Shaffer-Hartmann[6] method. β d-Mannoheptitol was recovered from the rats' urine and identified by its melting point. The results are set forth in Table I.

TABLE I.
Effect of Primulatol on the Glycogen Storage in the Livers of Rats.

Rat No.	Wt. before experiment gm.	Primulatol consumed gm.	Liver Glycogen %
Experimental			
2	113	2.8	.26
3	129	2.3	.11
4	122	2.0	.17
5	152	2.4	.07
6	149	2.5	.17
7	152	2.5	.10
8	134	2.5	.24
10	112	1.7	.10
			—
			Aver. .15
Controls			
11	122		.08
12	133		.21
13	126		.13
14	117		.16
15	128		.09
			—
			Aver. .13

Conclusion. β d-mannoheptitol unlike the hexahydric alcohol, mannitol, and the heptose, β d-mannoheptulose, is not capable of serving as a precursor of glycogen in the liver of the rat.

[4] Good, Kramer, and Somogyi, *J. Biol. Chem.*, 1933, **100**, 485.
[5] Pfluger, *Pfluger's Arch.*, 1906, **114**, 242.
[6] Shaffer and Hartmann, *J. Biol. Chem.*, 1920-21, **49**, 349.

9853

Effect of Addition of KCN to Whole Blood on Indophenol-reducing Power of Plasma.

GERALD J. FRIEDMAN, SAUL H. RUBIN AND WALTER KEES.
(Introduced by Elaine P. Ralli.)

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Pijoan, Townsend and Wilson[1] noted a considerable loss of vitamin C in blood serum done by the Farmer and Abt[2] method. Pijoan and Klemperer[3] reported that this loss could be prevented by the immediate addition of M/50 KCN to the blood. In order to determine the difference in the concentration of vitamin C in blood with and without KCN, the following experiments were done. Forty cc. of venous blood were drawn and placed immediately in an Erlenmeyer flask containing 8 drops of a 20% solution of potassium oxalate. The specimen was shaken gently and divided into two equal fractions, to one of which (B) were added 8 drops of 10% KCN resulting in an M/26 solution of KCN. Both fractions (A and B) were then immediately centrifuged and the plasma drawn off. The separated plasma from each fraction was divided into four 2 cc. portions. One of each of these samples (A_o and B_o) was precipitated immediately, using 2 cc. of plasma, 4 cc. of redistilled water and 6 cc. of a 10% solution of metaphosphoric acid. These samples were then centrifuged for 5 minutes and the filtrate decanted off. Three cc. samples of the filtrate were used for the titrations, which were done by the Farmer and Abt modification of the Tillman's method.[4] The remaining 6 plasma specimens (3 with KCN and 3 without KCN) were placed in the icebox and determinations of the vitamin C content were done after intervals of approximately 30, 90 and 160 minutes as shown in the chart. The first determinations, in this experiment, were done 45 minutes after the blood was drawn. This represents the maximum time required for preparing the filtrate; the average time taken in other experiments was 25 minutes.

Immediately after the first specimen of blood had been taken from the subject, 1000 mg. of vitamin C (Merck) were injected intra-

1 Pijoan, M., Townsend, S. R., and Wilson, A., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 224.

2 Farmer, C. J., and Abt, A. F., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 146.

3 Pijoan, M., and Klemperer, F., *J. Clin. Invest.*, 1937, **16**, 443.

4 Tillmans, J., Hirsch, P., and Hirsch, W., *Z. für Undersuch d. Lebensmitt.*, 1932, **63**, 1.

venously and after a period of 20 minutes a second 40 cc. sample of blood was drawn. In this way, it was possible to study the effect of KCN on blood from the same individual at high and low concentrations of the vitamin. This blood sample was also divided into 2 equal fractions (C and D) of 20 cc. each, to one of which (D) KCN was added in the same concentration as above. The plasma samples were treated in the same manner as specimens A and B and titrated at the same intervals of time. All plasma protein precipitations were done just prior to titration.

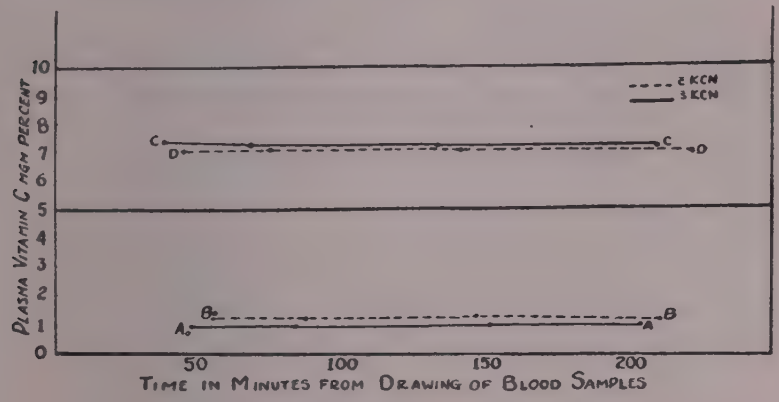

FIG. 1.

The results are shown graphically in the chart. At the low concentration of vitamin C, in the plasma to which KCN had been added (B), the indophenol-reducing power was equivalent to 1.13 mg. % of vitamin C. The sample to which KCN had not been added (A) had a concentration of 0.89 mg. %. Over the period of time observed (160 minutes) these concentrations showed little variation, remaining always within the experimental error of the method. At the higher concentrations (C and D), the dye reduction was equivalent to 7.39 mg. % in the specimen *without KCN* and 7.03 mg. % in the specimen to which KCN had been added. Again over the period of time observed (160 minutes) there was little change in the concentration of vitamin C at this level.

In a second experiment the effect of KCN (M/54) on the plasma vitamin C of another subject was determined at 4 different plasma levels, following the injection of crystalline vitamin C (Merck). The results were as follows (Table I).

This showed again the greater reduction of the dye by the plasma without cyanide at the higher vitamin C concentrations. The dif-

TABLE I.
Concentrations of Vitamin C mg. %.

Blood without KCN	1.42	2.92	5.40	8.16
Blood with KCN	1.88	2.88	5.14	7.54

ferences at the higher concentrations are consistent and are not due to experimental error.

These observations on the rate of destruction of the indophenol-reducing power of plasma at normal vitamin C levels do not agree with the findings of Pijoan, *et al.*,[1, 3, 5] who found an average half-life of about 60 minutes for vitamin C in separated plasma.

There is no evidence that the differences in reducing power observed in bloods at normal vitamin C levels are due to an inhibition of the oxidation of vitamin C. It may be that the greater reduction of the dye is due to the activation of some other redox system by the cyanide. It seems to us, on the basis of these observations, that there is no reason for the addition of KCN to blood for the determination of plasma vitamin C.

We are unable to explain the fact that at the higher levels of vitamin C KCN added to the blood actually resulted in a lessened reduction of the dye, an observation that we have confirmed repeatedly. Experiments bearing on this point are in progress.

9854

Longevity of Erythrocyte and Reticulocyte in Normal and Splenectomized Guinea Pigs.

ALBERT S. GORDON AND WILLIAM KLEINBERG. (Introduced by Harry A. Charipper.)

From Washington Square College, New York University.

We have employed the method of Escobar and Baldwin[1] to determine the longevity of the erythrocyte of the normal and splenectomized guinea pig. This consists in subjecting the animals to pressures sufficiently low to result in a significant increase in red cell count, and then recording the time necessary for the count to reach its original level. Use of this method has also been made for estimating the longevity of the circulating reticulocyte in such animals.

[5] Pijoan, M., and Eddy, E., *J. Lab. Clin. Med.*, 1937, **22**, 1227.

[1] Escobar, R. A., and Baldwin, F. M., *Am. J. Physiol.*, 1934, **107**, 249.

Young normal and splenectomized guinea pigs (weight 350-400 gm.) were placed in a specially constructed low pressure chamber and subjected for 10 days to pressures of 380 mm. Hg.* They were removed from the chamber once daily for about half an hour to clean the cages and replace the food. Red cell counts were made in duplicate from freely flowing blood drawn from the ear, each of us counting the cells on 400 squares of a Levy-Hausser haemacytometer. The counts were required to agree within 4%. Reticulocytes stained with brilliant cresyl blue were observed in wet mounts. At least 1000 erythrocytes were counted and the reticulocytes expressed as a percentage of these.

Representative results for 3 normal and 3 splenectomized guinea pigs are shown in Table I. The splenectomized animals were exposed to the low pressure stimulus 17 days after the operation which corresponds to the time when maximum effects following splenectomy are obtained.[2] The numbers of red cells are given in millions

TABLE I.

Days out of Chamber	Controls						Splenectomized					
	Reds			Retics.			Reds			Retics.		
	1	2	3	1	2	3	4	5	6	4	5	6
0	6.5	7.2	7.4	9.4	8.2	8.8	6.8	6.5	7.1	7.8	8.2	9.6
2	6.3	7.0	7.2	3.9	2.7	2.3	7.2	6.9	7.4	5.4	2.6	4.6
4	6.2	6.9	7.0	**0.4**	**0.3**	**0.5**	8.2	7.3	7.8	**0.6**	**0.7**	0.8
6	6.0	6.7	6.9	0.1	0.2	0.2	8.3	7.5	8.2	0.2	0.1	**0.4**
8	6.0	6.4	6.7	0.2	0.1	0.1	8.7	7.7	8.3	0.1	0.1	0.6
10	5.8	6.3	6.6	0.2	0.1	0.1	8.3	7.6	8.2	0.2	0.2	0.2
12	5.7	6.1	6.4	0.1	0.2	0.3	7.9	7.4	8.0	0.1	0.1	0.1
14	5.6	6.1	6.1	0.1	0.2	0.1	7.5	7.2	7.8	0.3	0.2	0.2
16	5.6	5.9	6.0	0.1	0.2	0.2	7.3	7.2	7.5	0.2	0.1	0.1
18	5.5	5.8	5.9	0.2	0.1	0.2	7.0	6.9	7.2	0.1	0.2	0.2
20	5.2	**5.6**	5.7	0.1	0.1	0.1	6.8	6.7	6.8	0.2	0.1	0.1
22	**5.0**		5.4	0.1		0.1	6.6	6.2	6.6	0.1	0.3	0.2
24			**5.2**			0.4	6.4	6.0	6.5	0.2	0.2	0.1
26							6.1	5.9	6.2	0.1	0.4	0.1
28							5.9	5.8	5.9	0.2	0.1	0.2
30							5.8	5.6	5.5	0.2	0.2	0.1
32							5.6	5.4	**5.2**	0.1	0.2	0.1
34							**5.4**	5.3		0.2	0.2	
36								**5.0**			0.1	

The numbers 1, 2, 3, 4, 5, 6, at the tops of the columns refer to the specific 6 animals whose red cell and reticulocyte counts are shown. 0 days out of the chamber refers to the counts taken immediately on withdrawal of the animals from the low pressure apparatus after 10 days' exposure. The normal counts are indicated in bold type on the days they are attained.

* Certain of our experiments with guinea pigs indicate that for this period of exposure to low pressures, erythropoietic hyperactivity ends almost immediately on removal of the animals from the chamber.

2 Gordon, A. S., and Kleinberg, W., *Am. J. Physiol.*, 1937, **118**, 757.

per cubic millimeter and the reticulocytes as a percentage of the total reds.

With this method, and taking into account the period of subjection to low pressures by adding on approximately half the number of days of exposure (as do Escobar and Baldwin), the longevity of the erythrocyte of 21 normal animals was found to range from 22-28 days. For 14 animals placed in the chamber 13-17 days after splenectomy, the longevity ranged from 32-38 days.† It will be seen from Table I that although the red cell counts in the control animals begin to fall almost immediately upon withdrawal of the stimulus, the counts in the splenectomized animals continue to rise for several days after interruption of the low oxygen tensions. These post-exposure rises in the splenectomized animals have been attributed to (1) the removal, with the spleen, of a large portion of the reticulo-endothelial system normally concerned with red cell phagocytosis and destruction, and (2) the increased resistance of the red cells following splenectomy.[2] The greater longevity of the splenectomized animal's erythrocyte is due, most likely, to the same factors. The longevity of the erythrocyte begins to diminish after about 30 days following splenectomy, with finally the value in the splenectomized animal becoming equal to that in the controls approximately 2½ months subsequent to spleen removal. This decrease in longevity of the splenectomized animal's erythrocyte is probably due to reticulo-endothelial compensation in other organs (*i. e.,* liver, lymph nodes, etc.) and to the gradual return of the red cell resistance to normal.[2]

The value for the longevity of the normal guinea pig erythrocyte obtained in this study corresponds closely to the one obtained by Escobar and Baldwin for man's erythrocyte. Since the normal red cell count of the animals employed in these experiments is approximately the same as man's normal count, this may be considered additional evidence in favor of Escobar and Baldwin's hypothesis that longevity is regulated to an extent by some relation between red cell population density and red cell fragility.

Table I shows that the longevity of the circulating reticulocyte is unaffected by splenectomy. In the course of studies, during the past few years, on approximately 150 animals, both normal and splenectomized, subjected to low pressures varying from 5 to 15

† Although a post-operative anemia which may last for 2 months develops in the splenectomized animals, it is possible to calculate the value of the "normal" count at any time after interruption of the stimulus from available extensive data on the course of this anemia.

days, which resulted in reticulocyte increases from 6% to 14%, the longevity of the reticulocyte was found to be remarkably constant. It ranged from 4 to 6 days. This value is in close agreement with those obtained by Heath and Daland[3] for the *in vitro* and *in vivo* maturation of reticulocytes of pernicious anemia patients.‡

Summary. 1. The red cell longevity in normal guinea pigs ranges from 22-28 days. 2. This value increases after splenectomy, reaching a maximum of 32-38 days approximately a month following the operation, and then falls off, becoming normal in about 2½ months. 3. The longevity of the circulating reticulocyte ranges from 4 to 6 days in both normal and splenectomized animals.

9855

Decreased Choline-Esterase Activity of Serum in Jaundice and in Biliary Disease.*

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In a previous communication,[1] the choline-esterase activity of human serum was determined by the Ammon method[2] with the reaction continuing over a 2-hour period at 30°C. Choline-esterase activity was expressed in cubic millimeters of CO_2 liberated, in a total volume of 2 cc., from 7.5 mg. acetylcholine chloride by 0.5 cc. of diluted serum (diluted 50 times). It was found that in a control group of 60 normal adults, the average reading was 67.6 mm.^{3} CO_2 liberated, and that all of the cases, except 7, fell between 44 and 80 mm.^{3} In a series of over 500 determinations in pathological conditions it was stated that in cases of jaundice there was a tendency toward depressed values.[1]

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[3] Heath, C. W., and Daland, G. A., *Arch. Int. Med.*, 1930, **46**, 533.

‡ The close correspondence between Heath and Daland's values for the *in vitro* maturation of reticulocytes and ours for the *in vivo* longevities would further support the contention that marrow hyperactivity ceases quite abruptly after withdrawal of the stimulus.

* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

[1] Antopol, W., Tuchman, L., and Schifrin, A., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 46.

[2] Ammon, R., and Voss, G., *Pfluger's Arch. f. d. ges. Physiol.*, 1935, **235**, 393.

TABLE I.
Choline-esterase Activity of Serum in Jaundice.

Case	Age	Diagnosis	Choline-esterase activity†	Van den Berg	Ieteric Index	Hb %	Patient Temp. °F	Ratio of esterified cholesterol to total cholesterol*
1	38	Cirrhosis of liver	10	1:67,000	14	63	99.4	40/250
2	56	" with hepatitis	13	1:250,000	27	85	98.6	30/205
3	62	Toxic hepatitis	15	1:17,000	35	70	101.	125/375
4	54	" " in pernicious anemia	18	——	20	98	100.2	61/200
					56		99.	
5	54	Cirrhosis of liver	19	1:500,000 (subicteric clin.)	2	96	98.6	50/200
6	59	" " " ; cholemia	20	1:100,000	12	86	98.6	46/315
7	48	" " " ; toxic hepatitis	22	1:100,000	14	46	99.8	——
8	13	Liver abscess; actinomycosis	24	1:50,000	17	78	99.	——
9	51	Acute hemolytic icterus	29	1:100,000	12	30	99.	trace/110
							103.	
10	23	Tertian malaria	30	1:80,000	15	75	98.	trace/85
11	63	Carcinoma of pancreas	31	1:60,000	23	70	100.	150
12	56	" " "	34	1:80,000	50	85	99.	140/270
13	42	Acute pancreatitis	38	1:14,000	40	—	102.4	——
14	46	Cholelithiasis; common duct stone	40	1:60,000	32	72	98.6	——
15	56	Chronic cholecystitis, cholelithiasis	46	1:250,000 (subicteric clin.)	27	78	98.6	——
16	50	Carcinoma of pancreas	48	1:17,000	42	85	98.6	315/515
17	71	Hypertension; cholecystitis, cholelithiasis	55	1:100,000	12	72	102.	——
18	26	Hepatosplenomegaly	57	——	36	75	99.	76/240
19	25	Catarrhal jaundice	63	1:80,000	13	80	98.6	105/200
20	8	" "	64	1:110,000	18	90	100.	25/200
21	17	Hodgkin's	76	1:70,000	20	90	99.	——

†Choline-esterase activity of serum expressed in mm^3. CO_2 developed in 2 hours at 30°C.
*Figures expressed in mg. %.

TABLE II.
Choline-esterase Activity of Serum in Biliary Disease.

Case	Age	Diagnosis	Choline-esterase activity†	Van den Berg	Icteric Index	Hb %	Patient Temp. °F	Ratio of esterified cholesterol to total cholesterol*
22	31	Liver abscess; actinomycosis	11	——	—	58	103.	——
23	66	Secondary anemia; cirrhosis of liver; heart failure	17	1:500,000	4	11	98.6	——
24	71	Acute cholecystitis	25	1:300,000	5	70	99.2	——
25	65	Splenic vein thrombosis	29	1:500,000	6	36	98.6	45/180
26	27	Bilharzia of liver	42	1:200,000	12	50	100.4	40/130
27	45	Chronic cholecystitis with cholelithiasis	48	1:500,000	8	85	99.	150/325
28	51	Essential hypertension; chronic cholecystitis with cholelithiasis	65	1:300,000	5	83	98.6	——

†Choline-esterase activity of serum expressed in mm^3. CO_2 developed in 2 hours at 30°C.
*Figures expressed in mg. %.

Twenty-one cases of jaundice (Table I) and 7 other instances of liver and biliary tract disease without jaundice (Table II) were investigated. Since fever and anemia also influence the choline-esterase activity,[1] we have tabulated the temperature at the time of drawing of the blood and have recorded the hemoglobin. It is obvious from these figures that there is a tendency to depressed values in cases of hepatic and biliary tract disease. The mechanism of this depression has not as yet been elucidated. However, a series of experiments which were suggested by these data showed that bile acids,[3] added to the reaction mixture in the form of their sodium salts, caused inhibition ranging from moderate effects to almost complete inhibition of enzymatic hydrolysis.

It was stated in the original publication that the choline-esterase activity in the serum may be a factor which is inversely related to the vagotonicity of the individual. In view of this, it is of interest to speculate on the relation of the depressed choline-esterase activity to the sweating, bradycardia, and fall in respiration, vagotonic symptoms which not infrequently occur in cases of hepatic disease with and without jaundice.

Summary. The choline-esterase activity of the serum in patients with jaundice or biliary tract disease was found to be depressed.

9856

Passage of Sulfanilamide from Mother to Fetus.

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Recent reports by Marshall and his associates[1, 2] indicate clearly that sulfanilamide when administered to animals and men is rapidly and uniformly distributed in different tissues. It is thus easy to conceive that the drug may also be detected in fetal circulation and amniotic fluid among pregnant animals. In order to substantiate this assumption, the following experiments were carried out.

Pregnant rabbits of not less than 3 weeks' duration were employed.

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3 Sobotka, H., and Antopol, W., *Enzymologica,* 1937, **4**, 189.

1 Marshall, E. K., Jr., Emerson, K., Jr., and Cutting, W. C., *J. A. M. A.*, 1937, **108**, 953.

2 Marshall, E. K., Jr., Emerson, K., Jr., and Cutting, W. C., *J. Pharm. and Exp. Therap.*, 1937, **61**, 196.

TABLE I.

Rabbit No.	Body wt., kg.	Dose, mg. per kg.	Duration of pregnancy, days	Interval between administration and sacrifice of animal, hrs.	Sulfanilamide, mg. %					
					Maternal Blood		Fetal Blood		Amniotic Fluid	
					Free	Total	Free	Total	Free	Total
1	3.20	420	32	3¾	15.7	26.5	14.6	18.1	†	†
2	3.60	375	33	5¼	13.7	24.7	12.1	16.7	†	†
3	3.60	375	31	5⅓	2.6	21.0	2.7	12.5	10.5	13.1
4	4.92	375	23	4⅔	9.8	20.2	6.2	†	†	†
5	3.49	375	23	5	6.3	35.2	6.4*	10.3*	2.2	†
6	4.15	375	26	5½	4.4	11.5	3.1	7.3	2.8	5.2
7	4.78	375	26	4¾	20.3	22.0	16.9	20.0	15.7	19.4

*Amount per 100 g. of entire fetal tissue.
†Not determined.

Sulfanilamide dispensed in capsules was given by mouth. The dose was 375 mg. per kg. in 6 animals, and 420 in the remaining one. After a period varying from 3¾ to 5½ hours during which active absorption took place, maternal blood samples were taken either from the marginal vein of the ear or by cardiac puncture. The animals were immediately sacrificed by a blow on the head, the amniotic fluid if sufficient was obtained by aspiration with a syringe, and the fetuses were freed from their placentae. In order to eliminate any possible contamination by the maternal blood, the fetuses were washed in running water and dried with a towel. After decapitation, the fetal blood was pooled and analyzed for sulfanilamide content. In one case no fetal blood could be collected because obviously the pregnancy was not fully advanced. It was therefore necessary to grind the fetuses with sand, and extract the residue with alcohol. All determinations of sulfanilamide both as the free and as the acetylated product were made with the colorimetric method perfected by Marshall and his coworkers.[3, 4]

The results are summarized in Table I. There is indeed no question that sulfanilamide passes from the maternal to the fetal circulation. The total amount expressed in mg. % is uniformly greater in the mother than in the fetuses, although the amount of free sulfanilamide appears to bear a close relationship between the maternal and fetal blood. The relatively low content of conjugated sulfanilamide on the fetal side might be due to less efficient mechanism of acetylation in the unborn animals. As in mature rabbits, sulfanilamide is probably also well distributed in fetal tissues as shown by animal numbered 5 in Table I. The presence of the drug in amniotic fluid is not unexpected since the fluid is generally considered to be derived from the maternal serum.

Summary. Sulfanilamide when given by mouth in pregnant rabbits has been shown to pass from the maternal to the fetal circulation. The acetylated form is relatively lower in the fetal blood than in the maternal blood. Sulfanilamide can be also detected in the amniotic fluid.

[3] Marshall, E. K., Jr., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 422.
[4] Marshall, E. K., Jr., *J. Biol. Chem.*, 1937, **122**, 263.

9857 P

Metrazol Convulsions and Their Relation to the Epileptic Attack.*

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The convulsions produced by the intravenous injection of metrazol (pentamethylentetrazol) as a therapeutic agent in schizophrenia, were recorded by means of normal and ultra-high-speed motion pictures and by electromyograms. The electromyograms of the activity of antagonistic muscles were recorded simultaneously by 2 matched amplifier systems which operated crystal actuated ink-writing recorders.

The typical metrazol seizure in man is composed of 3 stages: the first clonic stage, the tonic stage and the second clonic stage. The nature of the clonus is entirely different in the 2 clonic stages. As seen from the films and the electromyograms the clonus of the first clonic stage is the result of the alternating contraction of agonistic and antagonistic muscle groups; 2 to 4 of these 2-phase movements occurring per second. The second clonic stage is the result of an alternation between simultaneous contraction and relaxation of all muscle groups. It represents an alternating disappearance and reappearance of the continuous innervation which is present in the tonic stage. The change between contraction and relaxation is very rapid at the beginning of the second clonic stage, 12 to 14 contractions occurring per second. The movements gradually become slower and coarser. Towards the end of the second clonic stage only 1 to 2 contraction seizures occur per second and finally complete relaxation follows as is evidenced by a complete absence of muscle action potentials in the electromyogram.

The tonic stage, which in the electromyogram shows simultaneous high potentials in all muscles, represents a state similar to decerebrate rigidity. The movements of the second clonic stage correspond to an intermittent innervation of the same characteristics which were continuously present during the tonic stage. The second clonic stage, therefore, should be considered as an intermittent appearance of a state resembling decerebrate rigidity.

This explanation is in agreement with the fact that clonic con-

* This project was subsidized by a grant from the John and Mary Markle Foundation.

vulsions have often been observed in decorticated animals.[1-6] Hence, we may conclude that the second clonic stage occurs without any active participation of the cortex. The study of our motion picture records has demonstrated that the average major seizure[7] corresponds closely to the tonic and second clonic stage of the metrazol convulsion. Our electroencephalographic records show that action potentials of a distinct epileptic pattern can be obtained from the cortex during the tonic and following clonic stage.

This raises the question of whether these electroencephalograms are really the expression of some abnormal cortical activity which causes the epileptic attack or whether they are only an accompanying cortical phenomenon indicating that the cortex is influenced by the same abnormal activity of subcortical structure which is the basis of the epileptic attack. This question is of fundamental importance for all interpretations which have been or will be drawn from the use of electroencephalograms in the study of the pathophysiology of the epileptic attack. We are continuing our investigations of this question by recording simultaneously the movements and the electroencephalogram in patients and in animals having variously localized lesions of the brain, during metrazol seizures.

9858

Effect of 1,2,5,6-Dibenzanthracene on *Fusarium lini*.

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In addition to being able to initiate malignant growth, the carcinogenic hydrocarbons have been shown to stimulate cell division of certain microörganisms. Since it has been postulated that cancer may be associated with an abnormal glucose metabolism, it was

1 Marinesco, G., Sager, O., and Kreindler, A., *Revue Neur.*, 1932, **1**, 1329.

2 Muncie, W. S., and Schneider, A. J., *Bull. Johns Hopkins Hosp.*, 1928, **42**, 77.

3 Pike, F. H., and Elsberg, C. A., *Am. J. Physiol.*, 1925, **72**, 327.

4 Pike, F. H., Elsberg, C. A., McCulloch, W. S., and Chappell, M. N., *Arch. Neur. and Psychiat.*, 1930, **23**, 847.

5 Pike, F. H., Elsberg, C. A., McCulloch, W. S., and Chappell, M. N., *Am. J. Psychiat.*, 1930, **10**, 567.

6 Schoen, R., *Arch. f. exp. Path. u. Pharm.*, 1926, **113**, 257.

7 Wilson, S. A. K., *Handbuch d. Neurol.*, 1935, **17**, 1.

thought of interest to study the effect of a typical carcinogenic hydrocarbon on the growth and glucose utilization of *Fusarium lini*. This fungus has been shown to grow well on a medium composed of mineral salts and glucose as the only source of carbon producing a typical alcoholic fermentation.[1]

The carcinogenic hydrocarbon selected for this study was 1,2,5,6-dibenzanthracene. The culture medium had the following composition:

NH_4NO_3	1.00 gm.
$MgSO_4$	0.25
KH_2PO_4	0.50
Glucose	20.00
Distilled water to make 1,000 cc.	

Since in preliminary tests the above hydrocarbon had no effect on the growth of *Fusarium lini* grown on the above medium, it was felt that this might be due to the low solubility of the hydrocarbon in the medium. Therefore in the work here reported a water-soluble oxidation product of the hydrocarbon was used. This was prepared by a method similar to that of Boyland and Boyland[2] as follows:

A solution containing 0.123 gm. of 1,2,5,6-dibenzanthracene, dissolved in a small amount of anhydrous benzene, was exposed for 1¼ hours to the rays from a mercury arc ultraviolet light source. The solution was exposed directly to the light by being placed in a thin layer in one-half of a large Petri dish. From time to time benzene was added to replace that lost by evaporation. Finally the benzene was evaporated and the residue irradiated for 15 minutes. This residue was then extracted with several small portions of water, the washings filtered and the volume made up to 100 cc. This extract was used in the subsequent work.

A series of 16 Erlenmeyer flasks was prepared, each flask containing 100 cc. of medium. Eight of the flasks contained in addition one cc. of the water extract described above. After heat sterilization each flask was inoculated with a spore-mycelium suspension of *Fusarium lini*. The flasks remained at room temperature throughout the experiment. The flasks were arranged in pairs, with and without hydrocarbon. One pair was analyzed immediately for glucose by means of a saccharimeter. Sugar values are expressed as saccharimeter readings using a 400 mm. tube. At frequent intervals a pair of flasks was removed and the contents filtered through a Gooch crucible. A saccharimeter reading was taken on the filtrate,

[1] Anderson, A. K., *Minnesota Studies in Plant Science. Studies in the Biological Sciences*, 1924, No. 5, pp. 237-280.

[2] Boyland, E., and Boyland, M. E., *Biochem. J.*, 1934, **28**, 244.

the mycelium being washed, dried and weighed. In all, three series were run. Similar results were obtained in all series. The results of one series are shown graphically in Fig. 1.

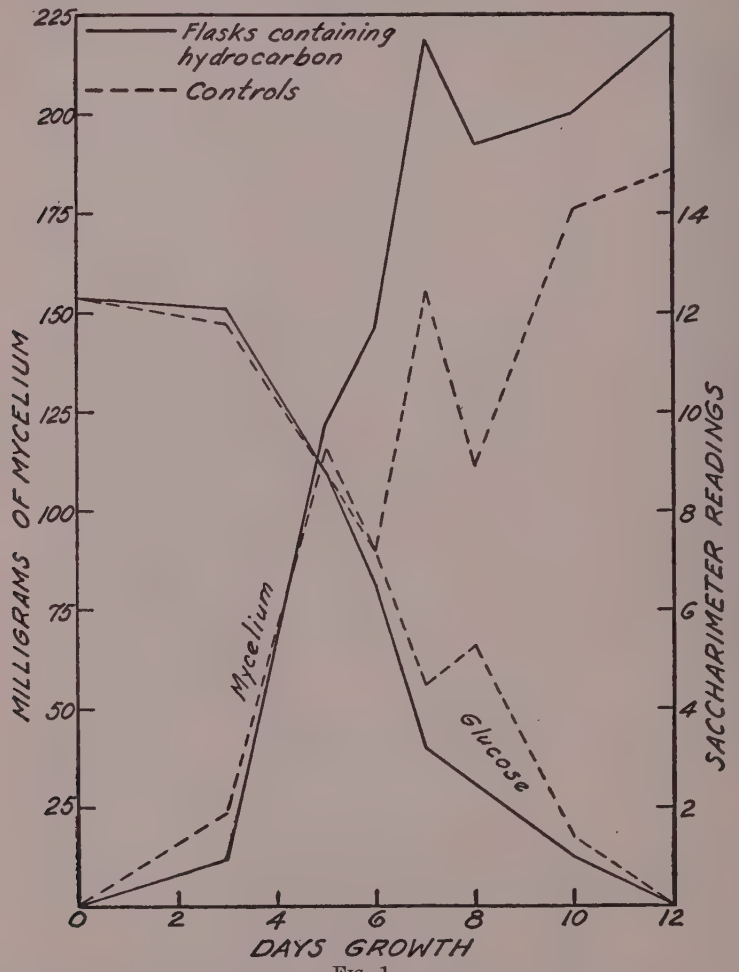

Fig. 1.

Showing mycelium production and glucose utilization of *Fusarium lini* as influenced by 1,2,5,6-dibenzanthracene.

An examination of the data presented shows that water-soluble derivatives of 1,2,5,6-dibenzanthracene after an initial inhibiting period were effective in stimulating the activities of *Fusarium lini* as evidenced by an increase in the weight of mycelium and a more rapid utilization of glucose.

9859

Limiting Content of Theophylline Necessary to Prevent Local Toxic Action of Mercurial Diuretics.

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In a previous paper[1] it has been shown that theophylline greatly diminishes the toxicity of Mercurin* and Salyrgan at the site of intracutaneous injection. The beneficial action of theophylline in enhancing absorption after intramuscular injection has also been demonstrated[2] and it was found[3] that one mole equivalent (3.88% of theophylline monohydrate) produces the maximum effect. Since this and other data[3] are strongly suggestive of compound formation between theophylline and mercurial diuretics of general structure $NaOCO\text{-}R^{II}\text{-}HgOH$ it seemed advisable to determine the limiting amount of theophylline necessary to prevent local toxicity.

The experimental procedure was the same as that described before[1] and consisted in the intradermal injection into the abdomen of the rabbit of 0.05 cc. of Mercurin and Salyrgan to which varying amounts of theophylline had been added. Each solution was prepared to contain 39 mg. of mercury per cc. (about 0.2 M) and its pH was accurately determined with the glass electrode.

In the accompanying photograph, taken 24 hours after injection, the mole equivalents of theophylline and pH values for each of the 7 solutions are shown adjacent to the corresponding sites of injection. In all 5 rabbits were injected in this way for Mercurin and 3 for Salyrgan. The results for the 2 drugs are very much alike and were found to be so consistent that only one photograph is shown. It will be seen that the local toxicity decreases in proportion to the amount of theophylline present and in the case of the solutions containing 1, 1.25, and 1.5 mole equivalents, so little reaction was produced that it is difficult to locate exactly the sites of injection.

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1 DeGraff, A. C., and Batterman, R. C., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 1546.

* The sodium salt of N(γ-oxymercuri-β-methoxypropyl)-d-α-camphoramic acid. Mercupurin is a 10% solution of this salt which is also 5.35% with respect to theophylline monohydrate. The Mercurin used in suppositories contains 20% of the free acid.

2 DeGraff, A. C., Batterman, R. C., and Lehman, R. A., *J. Pharm. Exp. Therap.*, 1938, **62**, 26.

3 Lehman, R. A., and Dater, A., *J. Pharm. Exp. Therap.*, in press.

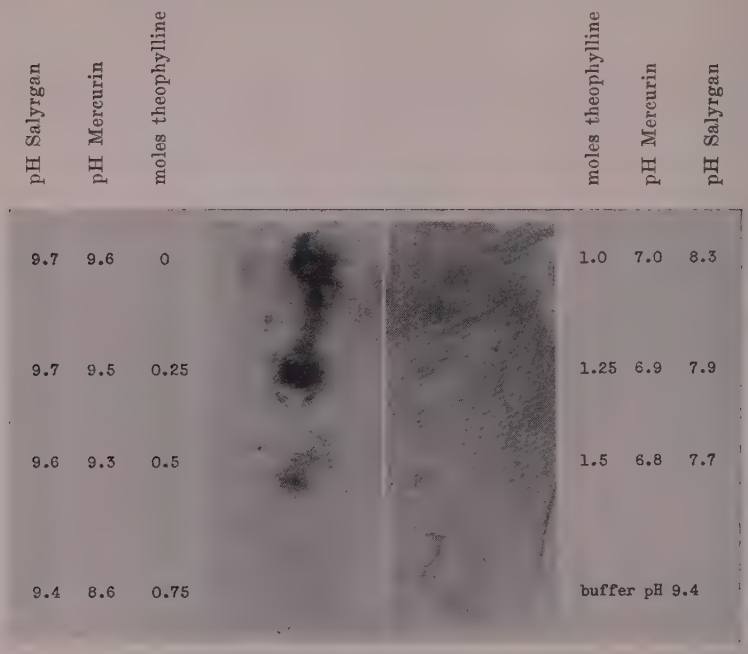

FIG. 1.

Photograph showing the influence of increasing amounts of theophylline upon the local lesions produced in the skin of the rabbit by Mercurin and Salyrgan.

Since theophylline reduces the pH of these solutions of diuretics a buffer was also injected as a control but in no case was an appreciable reaction observed.

One mole equivalent has been found to be the limiting amount of theophylline necessary to prevent the local toxic reaction to Mercurin and Salyrgan. The results are in agreement with other evidence pointing to compound formation between theophylline and mercurial diuretics.

9860

Sulfone and Sulfonanilide Therapy in Streptococcal Infections.

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From the Institute of Pathology, The Western Pennsylvania Hospital, Pittsburgh, Pa.

Recent reports[1,4] have indicated that 4,4′-di-(acetylamino)-diphenylsulfone and 4,4′-diamino-benzenesulfonanilide are high in chemotherapeutic efficacy and relatively low in toxicity.

In order to contribute further data on these points, the two above mentioned drugs* were tested parallel with sulfanilamide against experimental hemolytic streptococcal infections of mice.

All animals were infected intraäbdominally with 0.5 cc. of various dilutions (10^{-8}, 10^{-6}, 10^{-4}, and 10^{-2}) of 18-hour broth cultures of strain C 203.[5,6,7] However, a different culture was used for each experiment. Each dilution involved the use of 40 to 45 mice divided into equal groups: one control and 3 treated. In one experiment (Fig. 1 A) there were only 2 treated groups.

Oral treatment was begun 2 to 4 hours after infection and continued daily as indicated in Fig. 1. All chemicals were suspended in 15% aqueous gum acacia; the sulfone as 2.5 and 5% suspensions, and the anilide as well as the sulfanilamide as 5% suspensions. Because the anilide proved unstable in suspension, the latter was freshly prepared prior to each treatment. The therapy was continued for 10 days except in the case of the smallest infecting dose where it was discontinued after 5 days.

The therapeutic results were good with all 3 drugs (Fig. 1 A and B) when the infecting dose was low or moderate (10^{-8} to 10^{-4} dilution of the culture). However, in one of 2 experiments performed at different times, each involving the use of a heavy in-

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1 Whitby, L. E. H., *Lancet*, 1937, **1**, 1517.

2 Fourneau, E., Tréfouël, J., Tréfouël, J., Mme., Nitti, F., and Bovet, D., *Compt. rend. Acad. d. sc.*, 1937, **205**, 299.

3 Fourneau, E., Tréfouël, J., Tréfouël, J., Mme., Nitti, F., and Bovet, D., *Bull. Acad. de méd.*, Paris, 1937, **118**, 210.

4 Bauer, H., and Rosenthal, S. M., *Pub. Health Rep.*, 1938, **53**, 40.

* These chemicals were synthesized and donated to us by the Monsanto Chemical Company of St. Louis, Missouri.

5 Long, P. H., and Bliss, E. A., *J. A. M. A.*, 1937, **108**, 32.

6 Raiziss, G. M., Severac, M., and Moetsch, J. C., *J. Chemotherapy*, 1937, **14**, 1.

7 McKinney, R. A., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 33.

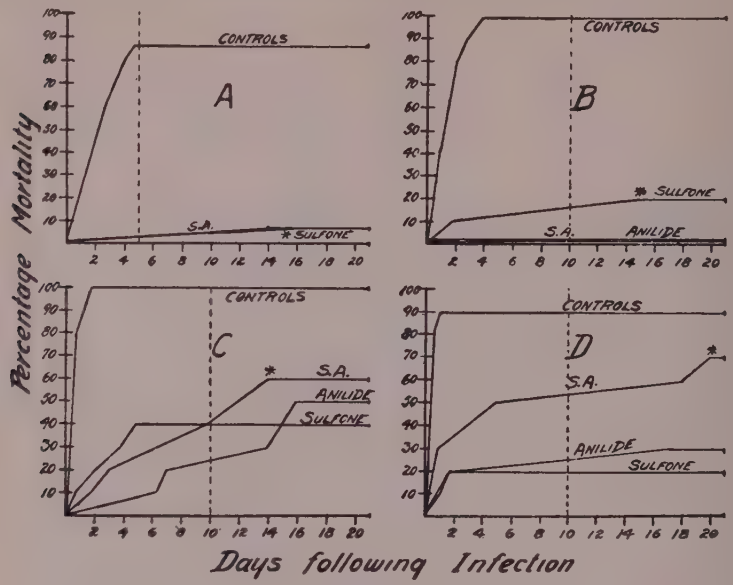

FIG. 1.

A: Culture diluted 10^{-8}
14 mice per group
Daily *Dosage* (oral) mg.
Sulfanilamide 10
Sulfone 5

B: Culture diluted 10^{-4}
10 mice per group
Daily *Dosage* (oral) mg.
Sulfanilamide 10
Anilide 10
Sulfone 5

C: Culture diluted 10^{-2}
10 mice per group
Daily *Dosage* (oral)
Sulfanilamide 10
Anilide 10
Sulfone 5

D: Culture diluted 10^{-2}
10 mice per group
Daily *Dosage* (oral)
Sulfanilamide 10
Anilide 10
Sulfone 10

All mice injected with 0.5 cc. of diluted culture, intraäbdominally. The vertical broken lines indicate cessation of therapy.
S.A.: Sulfanilamide.
*Death not due to streptococcal infection.

fecting dose (10^{-2} dilution of the culture), somewhat divergent results were obtained. Although both, the anilide and the sulfone, showed a suggestion of superiority over sulfanilamide (Fig. 1 C), a more definite indication of this was shown in the other experiment (Fig. 1 D), where all dosages were equal.

The difference in the results of the anilide therapy in these 2 experiments (Fig. 1 C and D) is perplexing because other conditions were so nearly alike. In C (Fig. 1), 3 out of 5 deaths in the anilide group occurred 14 to 17 days after infection, whereas in D (Fig. 1), this group had only 3 deaths, of which one occurred after the second day.

The livers and kidneys of some of the treated mice from each group were examined histologically for lesions attributable to the medications, but all changes found in the various treated animals were also represented in the untreated controls.

Attempts to determine the maximum tolerated single oral dose of the sulfone were discontinued when it was found that 20 gm. mice tolerated 400 mg. of the drug without the slightest untoward effect. Similarly, 100 mg. were tolerated subcutaneously. The latter injections, however, resulted in firm, subcutaneous nodules which after 2 weeks consisted essentially of chalky white deposits of sulfone crystals. Microscopically, in 24 to 48 hours following such injection, there was marked local edema with moderate leukocytic exudation in the region of the crystalline sulfone deposits.

The anilide was somewhat more toxic than sulfanilamide. Four out of 5 mice which received 100 mg. of the anilide by mouth died, whereas of 5 mice receiving 80 mg. sulfanilamide, none died; and of 4 mice receiving 120 mg. sulfanilamide by mouth, only one died. Subcutaneously, mice tolerated 60 mg. of the anilide without symptoms except for the development of a small, firm nodule at the site of injection.

The sulfone appeared to be more effective than the anilide against infections in mice caused by the C 203 strain, and because of the extremely low toxicity the sulfone appears to hold more promise of finding practical application.

Our results are in general agreement with the conclusions drawn by Bauer and Rosenthal.[4]

Conclusions. 1. 4,4'-di-(acetylamino)-diphenylsulfone is more efficacious than sulfanilamide against certain hemolytic streptococcal infections of mice. 2. Against these same infections, the 4,4'-diamino-benzenesulfonanilide is as good as or better than sulfanilamide. 3. The sulfone is less toxic than either the anilide or sulfanilamide. 4. Medication of mice with these drugs produced no demonstrable hepatic or renal lesions.

9861

Studies in Chemotropism. Source of Substances Attracting Polymorphonuclear Leukocytes to Bacteria.*

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One of the outstanding phenomena in bacterial infections is the migration of polymorphonuclear leukocytes into the infected area. This leukocytic reaction, known as chemotropism or chemotaxis, is presumably brought about by the release of some substance in the infected area; but what this substance is, whether it comes from bacteria or from injured cells of the host or from both, is uncertain.

It seemed probable from previous experiments[1] that the source of the attractive substance is, at least in part, the bacteria themselves. The present experiments were designed to test this hypothesis more rigorously.

In infections *in vivo*, products of cell- and tissue-injury cannot be excluded, but *in vitro*, experiments can be planned in such a way that the only possible source of attraction is the bacteria. If, under these conditions, leukocytes show positive chemotropism, we would conclude that bacteria do indeed give off attractive substances.

In the present experiments a clump of *Staphylococcus albus* was taken from an agar slant and placed on a glass slide, where the bacteria formed a small circle 0.1 to 0.2 mm. in diameter. The leukocytes were obtained by injecting 150 cc. of isotonic NaCl into the peritoneal cavity of the rabbit. The solution was withdrawn after 4 hours and leukocytes were concentrated by light centrifugation. The cells were then suspended in plasma of the same animal.

Since the purpose of these experiments was to find out whether leukocytes show chemotropism to bacteria in the absence of injured tissue-cells, it was essential to prevent any of the leukocytes from coming into contact with and phagocytizing the bacteria, because thereby the leukocytes might be injured and give off substances that would attract other leukocytes.[2] The problem, therefore, was to isolate the bacteria from the leukocytes. This was done by plac-

* This investigation was aided by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

1 McCutcheon, M., and Dixon, H. M., *Arch. Path.*, 1936, **21**, 749.

2 McCutcheon, M., Wartman, W. B., and Dixon, H. M., *Arch. Path.*, 1934, **17**, 607.

ing a minute drop of liquid plasma (without cells) on a coverglass and superimposing this drop on the bacteria, so that the plasma formed a disk with the clump of bacteria in the center. After this drop of plasma had coagulated, a suspension of leukocytes was allowed to run in between slide and coverslip, filling the space except that already occupied by the coagulated plasma. The preparation was sealed with petrolatum and observed with the microscope, at 37°C.

A representative experiment is illustrated in Fig. 1. By means of a drawing-ocular, a microscopic field was projected on a piece of paper and the outline of the bacterial clump was recorded. The broken line indicates the interface between the circle of plasma originally free from leukocytes, to the right, and the plasma containing leukocytes, to the left. The position of each leukocyte at the first observation, taken about 15 minutes after making the preparation, is indicated by an open circle. It is seen that 2 leukocytes had already moved into the inner ring of plasma. The path of each cell was now recorded for 15 minutes. During this time, all but one leukocyte moved closer to the bacteria.

The method used for evaluating chemotropism has been described in an earlier paper.[3] It consists in determining how nearly straight a cell moves toward a source of attraction. The cell designated "A" in Fig. 1 will be used as an illustration. At the first observation this cell was 123 microns from the edge of the bacterial clump, at the last observation, 78 microns. The difference, 45 microns, is divided by the length of the actual path which the leukocyte travelled in the same time. This was 75 microns. The quotient +0.6, is the value of chemotropism. If the cell had approached the bacteria in a straight line the value would have been +1, which is the maximal positive value of chemotropism; if it had moved directly away, the value would have been −1, *i. e.*, maximal negative chemotropism.

The average value for the 17 cells shown in Fig. 1 is +.65, a fairly high value. In 9 similar preparations, positive chemotropism was shown in each case, these observations being made before any leukocyte had reached the bacteria. Combining the results of all 10 experiments, the value of the mean and standard deviation for 184 leukocytes was $+0.53 \pm 0.33$. Nine control preparations, made in the same way except that bacteria were omitted, showed only slight positive chemotropism; for 162 cells the mean and standard deviations were $+0.15 \pm 0.42$.

From these values we conclude that leukocytes were attracted,

[3] Dixon, H. M., and McCutcheon, M., *Arch. Path.*, 1935, **19**, 679.

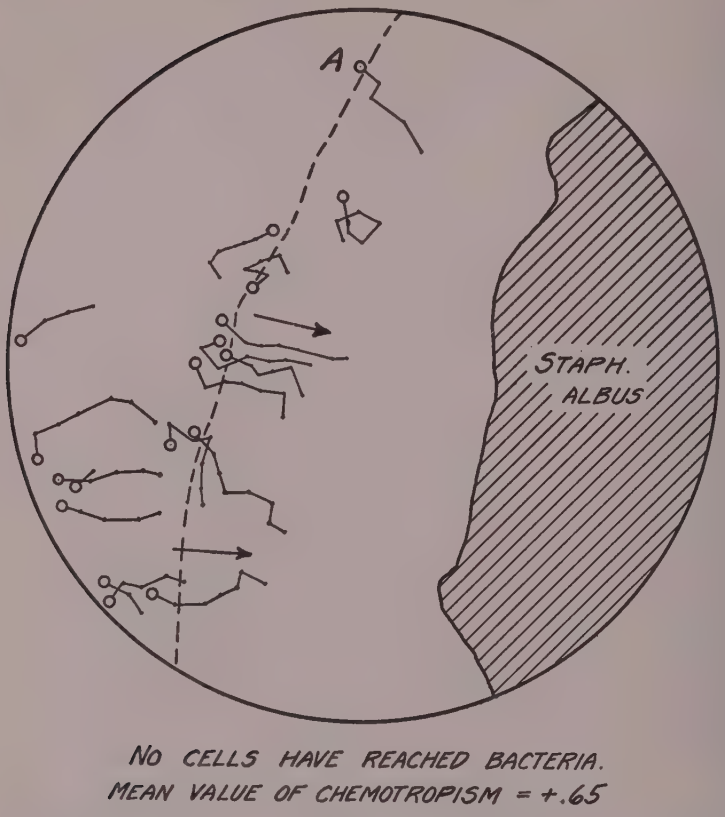

FIG. 1.

Camera lucida record of the paths of 17 leukocytes observed for 15 minutes, before any cells had reached the bacteria. The cells with one exception are moving toward the bacteria. The mean value of chemotropism is +0.65.

usually strongly, by *Staph. albus* even when no products of tissue-injury were present. This result is consistent with the hypothesis that the attractive substance is derived from the bacteria themselves.

It was now of interest to find out whether chemotropism would be increased after some of the leukocytes had reached the bacteria and had begun to phagocytize them. It seemed possible that phagocytes, gorged with bacteria, would suffer injury and give off chemotropic substances, which would increase the effectiveness of the bacterial products. Therefore the following additional observations were made on the same 10 preparations used in the experiments reported above. After phagocytosis had begun, the paths of other leukocytes were analyzed as they approached the bacteria. The mean and standard deviation for 260 cells was $+0.51 \pm 0.34$, a value

that is not significantly different from that obtained before any leukocytes reached the bacteria. Therefore phagocytizing leukocytes did not increase the chemotropic effect of the bacteria, presumably because the leukocytes were not injured.

We conclude that in these experiments the source of attraction was the bacteria themselves. The nature of the attracting substance and the possibility that attraction by bacteria may be due in part to substances adsorbed from the culture-medium are now being investigated.

Though in these experiments we obtained chemotropism in the absence of damaged tissue-cells, yet under other conditions, products of tissue-injury are important sources of chemotropism (the literature has been reviewed by Silverman[4]).

Summary. Experiments were designed to show whether bacteria chemotropically attract leukocytes directly, or only indirectly as the result of injuring cells and tissues and causing these to liberate chemotropic substances. Clumps of *Staph. albus* on a glass slide were separated from a suspension of rabbit's leukocytes by a zone of cell-free plasma. Leukocytes moved into the inner ring of plasma and made their way to the bacteria, showing strong positive chemotropism. Values of chemotropism obtained before any leukocytes reached the bacteria represent the reaction to bacteria alone, no tissue-cells being present in the clump of bacteria. After a number of leukocytes reached the bacteria and began to phagocytize them, the value of chemotropism was determined for other leukocytes *en route* to the bacteria. The two values of chemotropism were not significantly different. It is concluded that leukocytes may react chemotropically to substances given off directly by bacteria.

9862

Action of Pentamethylenetetrazol (Metrazol) on Splanchnic Circulation of the Dog.

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The analeptic drug pentamethylenetetrazol (Metrazol) used in the treatment of cardiovascular conditions has been reported[1] to

[4] Silverman, D., *Arch. Path.*, 1938, **25**, 40.

[1] Camp, W. J. R., *J. Pharm. and Exp. Therap.*, 1928, **33**, 81.

produce dilatation of the abdominal capillaries. Because of the extensive clinical use of this drug,[2] it was felt desirable to restudy its action on the intact splanchnic vascular bed of the dog.

For this study we used 23 female dogs, the average weight being 7 kg. The anesthetic employed was chloralosane—80 mg. per kg. injected intravenously. Blood pressure and respiration were recorded in the usual manner. Oncometers were placed on the spleen and small intestine or a loop of ileum was exteriorized and kept in a chamber containing normal saline at body temperature. The mesentery of this loop was transilluminated from below and the vessels were observed through a lens system whose magnifying power could be varied at will.

Following a control period with observation on the blood vessels of an intestinal loop, the drug was injected intravenously as a 10% solution. The dosage ranged from 0.25 cc. to 2.0 cc. per injection and each animal received several injections during a period of 2 to 3 hours. In some instances the same dose was employed throughout a single experiment; in others, the dosage was varied. Almost continuous observations were made of the caliber of the large and small arteries, the arterioles and venules and the large and small veins. Any changes noted were correlated with the normal variation as established before the administration of the drug.

In a series of 12 animals, the predominant effect of the drug on the entire splanchnic circulation was constriction in 3 cases and dilatation in 3 others. There was no significant effect in the remaining 6 dogs.

In another group of 7 dogs, shock was induced by the intravenous injection of peptone (2 cc. per kg. of a 10% solution). Metrazol administered during the period of profound shock apparently produced very minimal splanchnic vasoconstriction with slight transient elevation of blood pressure in 2 dogs, but had no significant effect on the other 5 dogs.

Although the predominant effect in a given animal was constriction or dilatation, there was no uniformity in response in the same animal to several injections. Thus, after the first dose, a large artery might constrict while after the second dose of the same amount, it might dilate. Also constriction of the arteries might be accompanied by venous dilatation, or *vice versa*. At times, an injection of 15 mg. per kg. would produce convulsions and it appeared as though the vascular reactions were associated with the convul-

[2] *New and Non-Official Remedies*, 1937, 301.

sions. To establish the rôle played by convulsions, strychnine sulphata (0.05 cc. of a 1% solution per kg.) was given to 3 dogs, with results on the splanchnic vascular bed similar to those found in the cases of metrazol with convulsions.

In 4 other dogs the effect of pentamethylenetetrazol on the volume of the spleen and small intestine was ascertained. As shown in Table I, the changes in both viscera were negligible until convulsions appeared.

TABLE I.

	Dose, cc.	Time	Convulsions	Spleen Volume Change
Dog 9B	0.1	9:50	0	0
Wt. 8 kg.	0.1	10:15	0	0
	0.2	10:40	0	0
	0.5	11:00	0	0
	1.0	11:30	++++	Reduced +++
Dog D	0.25	9:55	0	0
Wt. 7 kg.	0.5	10:20	0	0
	1.0	10:40	twitches	0
	1.0	11:15	+++	Reduced +

From this work, it appears that there is no significant or consistent effect of pentamethylenetetrazol on the splanchnic vascular circulation or on the blood pressure of the anesthetized dog. The slight changes observed may well be due to reflexes resulting from increased respiration or muscular activity associated with convulsions rather than to any direct action of the drug on the peripheral vessels.

9863

Pediculosis in Rats Kept on a Riboflavin-Deficient Diet.

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In the course of experiments on the systematology of the vitamin B_2 complex, about 600 rats kept on diets deficient in riboflavin[1] have been observed and about 2000 rats kept on diets deficient in

[1] György, P., *Nature*, 1934, **133**, 498; *Biochem. J.*, 1935, **29**, 741.

vitamin B_6[1, 2] and related factors, such as the factor affecting maturation of cells in the bone marrow[3] and the filtrate factor.[4]

Lack of vitamin B_6 in the diet becomes manifest in rats in the form of the specific clinical picture of "acrodynia," and deficiency of the maturation factor in the form of panmyelophthisis. Riboflavin deficiency, on the other hand, is characterized mainly by stunted growth and only in some experimental animals by loss of fur and by small yellow scales.[5] Under the conditions chosen it was not possible to confirm the findings of Day and his coworkers[6] concerning the high incidence of cataract in rats kept on diets deficient in riboflavin.

During the study of the vitamin B_2 complex in the last 5 years, pediculosis has been encountered exclusively in rats fed diets deficient in riboflavin. In these diets vitamin B is represented only by B_1, and the vitamin B_2 factors other than riboflavin are provided by Peters' eluate.[7] At first, pediculosis was regarded as a purely accidental and unspecific finding, one possibly related to a generally diminished vitality in these rats, which would then be unable to keep themselves clean in the efficient manner of normal animals. To our surprise, however, the majority of the rats with pediculosis were by no means weakened and inactive. Moreover, pediculosis was not observed even in moribund rats ill with acrodynia (B_6 deficiency) or with progressive panmyelophthisis caused by deficiency of the maturation factor. In the rats dying from panmyelophthisis there was prolonged agony, with complete muscular inactivity, and no appreciable intake of food for several days.

Intercurrent diseases, such as pneumonia, lung and kidney abscesses, generalized sepsis and diarrhea, which were frequently observed in this series of vitamin B_2 studies, did not apparently prepare the way for the occurrence of pediculosis except, again, in the rats fed riboflavin-deficient diets. It should be pointed out, however, that secondary infections of this kind did not usually appear in rats that exhibited pediculosis at the time they were on the diets devoid of riboflavin.

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2 György, P., *J. Nutrition*, in press.

3 György, P., Goldblatt, H., Miller, F. R., and Fulton, R. P., *J. Exp. Med.*, 1937, **66**, 579.

4 Lepkovsky, S., and Jukes, T. H., *J. Biol. Chem.*, 1936, **114**, 109.

5 György, P., Sullivan, M., and Karsner, H. T., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 313.

6 Day, P. L., and Langston, W. C., *J. Nutrition*, 1934, **7**, 97.

7 Kinnersley, H. W., O'Brien, J. R., Peters, R. A., and Reader, V., *Biochem. J.*, 1933, **27**, 225.

Experimental conditions with regard to kind of cages, basal diet, care, location in the animal room and seasonal factors were practically identical for the rats on diets deficient in riboflavin and those fed diets devoid of vitamin B_6 and related factors.

Pediculosis became apparent in about 20% of the animals that were kept for a sufficient length of time (more than 8 to 10 weeks) on the riboflavin-free diet. Therapeutic administration of riboflavin was followed not only by growth in the rats but also by complete disappearance of the pediculi, often accompanied by replacement of the diseased patches by new fur.

That it is possible to cure pediculosis in rats by nutritional means is in itself a significant finding and adds further weight to the conclusion that pediculosis and riboflavin deficiency in rats are interrelated.

Summary. Chronic riboflavin deficiency in rats is often accompanied by pediculosis. This disease has never been observed in rats manifesting symptoms of a deficiency of other factors of the vitamin B_2 complex. Administration of riboflavin by mouth has a curative effect on this type of pediculosis in rats.

9864 P

Treatment of Spontaneous Canine Distemper with Sulfanilamide.*

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Para-aminobenzene sulfonamide (sulfanilamide) and disodium 4-sulphamido-phenyl-2-azo-7-acetylamino-1-hydroxynaphthalene 3, 6-disulphonate (prontosil) have been used successfully in the treatment of beta hemolytic streptococci infections, and more or less good results have been reported on the use of these compounds in the treatment of meningococcal, pneumococcal, and gonococcal infections, gas gangrene, and as a urinary antiseptic.

Carré[1] reported that distemper in dogs is caused by a filtrable virus and this work was subsequently corroborated in carefully

* Aided by a grant from John Roesing and the Oscar Aberle Fund.

1 Carré, H., *Compt. rend. Acad. d. Sc.*, 1905, **140**, 689, 1489.

controlled experiments, by Dunkin and Laidlaw.[2] Our experience with canine distemper and a large number of autopsies with bacterial cultures of the organs led us to believe that in many cases a superimposed infection was more fatal than the virus infection itself. In most instances this superimposed infection is due to streptococcus, staphylococcus, pneumococcus, and the bacillus bronchiseptus. Since in many of our fatal cases streptococci were the prevailing organisms, an attempt seemed justified to treat distemper with sulfanilamide. It has been our experience that the mortality of dogs with distemper varies between 40-100% in normal dogs and between 90-100% in postoperative dogs.

The virulence of the virus seems to undergo periodic variations; therefore we are reporting 2 series of dogs with distemper, kept in our laboratory during the winter of 1937-38. One group was not treated and served as control; the other group was treated with sulfanilamide or prontosil.

Sulfanilamide was given by mouth in doses of 0.33 gm. t.i.d. and later by subcutaneous injection of 100 cc. of a 1% solution in saline with 5-10% glucose when necessary, b.i.d. Prontosil† was administered intramuscularly, 1 cc. per kg. body weight, of the 5% solution b.i.d.

The clinical signs of distemper are a warm, dry nose, fever, apathy, loss of appetite, serous and later purulent nasal discharge, conjunctivitis, loss of weight, anemia, leucocytosis and frequently, diarrheas. Treatment was instituted when nasal discharge was observed and continued until it ceased; this period varied from 3 days in the mild cases to 18 days in the very severe ones. Relapses occurred in all cases where treatment was discontinued before cessation of nasal discharge The success of the treatment was usually noticeable on the third day: appetite returned, the apathy diminished, the low red blood count and hemoglobin rose, while the high white blood count began to return to normal.

The single fatality in the group of treated animals was due to

TABLE I.
Comparison of Untreated and Treated Groups of Dogs with Distemper.

	Controls			Treated		
	Stock	Operated	Total	Stock	Operated	Total
No. of Dogs	13	7	20	7	10	17
Mortality	13	5	18	1	0	1

2 Dunkin, G. W., and Laidlaw, P. P., *J. Comp. Path. and Therap.*, 1926, **39**, 201, 213.

† Prontosil was generously supplied by the Winthrop Chemical Company.

the intentional interruption of therapy before cessation of nasal discharge. The lower mortality of the operated dogs in both groups may be attributed to the careful postoperative treatment which they received.

Since the above treatment was instituted as a routine measure the previously high incidence of distemper in our animal house has dropped so rapidly we feel confident that we are able to stamp out epidemics of distemper among our dogs. We would like to mention that many stock dogs appear perfectly well, yet following an operation they will often develop a fatal case of distemper.

Autopsies. From the lungs of untreated dogs all or some of the above named pathogenic organisms could be cultured in most instances. In the case of our single fatality in the treated series a nonhemolytic streptococcus was found in cultures from the lungs. Two other dogs which were cured were sacrificed 5 and 9 days respectively after clinical recovery. In the first, extensive consolidation was found in both lungs but no organism could be cultured. This animal had not been able to regain strength as the other treated dogs did. In the second dog, the lungs were normal and no other pathology was noticed.

One of the dogs who had been cured of distemper was operated upon 18 days afterwards, and then had a mild attack, possibly a relapse, of distemper, which was cured in the course of 5 days.

In 2 dogs recovered with therapy nervous tics were observed, similar to those we sometimes see in animals cured spontaneously or by treatment with distemper immune serum. We therefore believe that the tic was not due to the drugs used, but to central nervous involvement observed in distemper. So far we have not been able to find evidence of disorders to the dogs following the above therapy. Sulfanilamide by subcutaneous injection (in saline and with glucose) was preferred in cases when the dog refused food and water. Prontosil had the advantage of easier administration. We feel justified to publish this preliminary report in order to draw attention of other laboratories to this effective method of treatment. At the conclusion of our work our attention was called to the fact that Dochez and Slanetz[3] succeeded in curing distemper in ferrets and dogs with sodium sulfanilyl sulfanilate.

Summary and Conclusions. Sulfanilamide and prontosil have been used successfully in the treatment of distemper in dogs. Both drugs seem to be equally effective.

We are obliged to the Department of Bacteriology of Michael Reese Hospital for advice and help.

[3] Dochez, A. R., and Slanetz, C. A., *Science*, 1938, **87**, 142.

9865

Induced Postpartum Lactation in Hypophysectomized Rats.*

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Although lactation has been induced in the hypophysectomized, postpartum guinea pig by injecting crude pituitary extracts, or purified lactogenic hormone supplemented by salt, cortin or adrenocorticotropic hormone,[1-5] Nelson and Gaunt using similar methods failed to maintain lactation in hypophysectomized, lactating rats.

To obtain further evidence on this point 14 rats were hypophysectomized 5-24 hours before delivery. Four of these rats were retained as controls, and the remaining 10 were injected twice daily with relatively large subcutaneous doses of a lactogenic preparation containing adrenocorticotropin, but no thyrotropin, gonadotropin or somatotropin. All rats were given 1.0 cc. of a 20% glucose solution twice daily.

Four rats received a total dose of 0.5 gm. of a sheep pituitary extract equivalent to approximately 2,500 pigeon macro-units of mammotropin[6] and 10 rat units of adrenocorticotropin,[7] over a period of 10 days. The young, although nursed, did not obtain

* Aided by grants from the Board of Research of the University of California and the Rockefeller Foundation, New York City, administered by Professor Herbert M. Evans.

1 Gomez, E. T., and Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 404.

2 Nelson, W. O., and Gaunt, R., *ibid.*, 1936, **34**, 671.

3 Gomez, E. T., and Turner, C. W., *ibid.*, 1936, **35**, 365.

4 Gomez, E. T., and Turner, C. W., *ibid.*, 1937, **36**, 78.

5 Nelson, W. O., and Gaunt, R., *ibid.*, 1937, **36**, 136.

6 Lyons, W. R., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 645.

7 Moon, H. D., *ibid.*, 1937, **35**, 649.

FIG. 1.

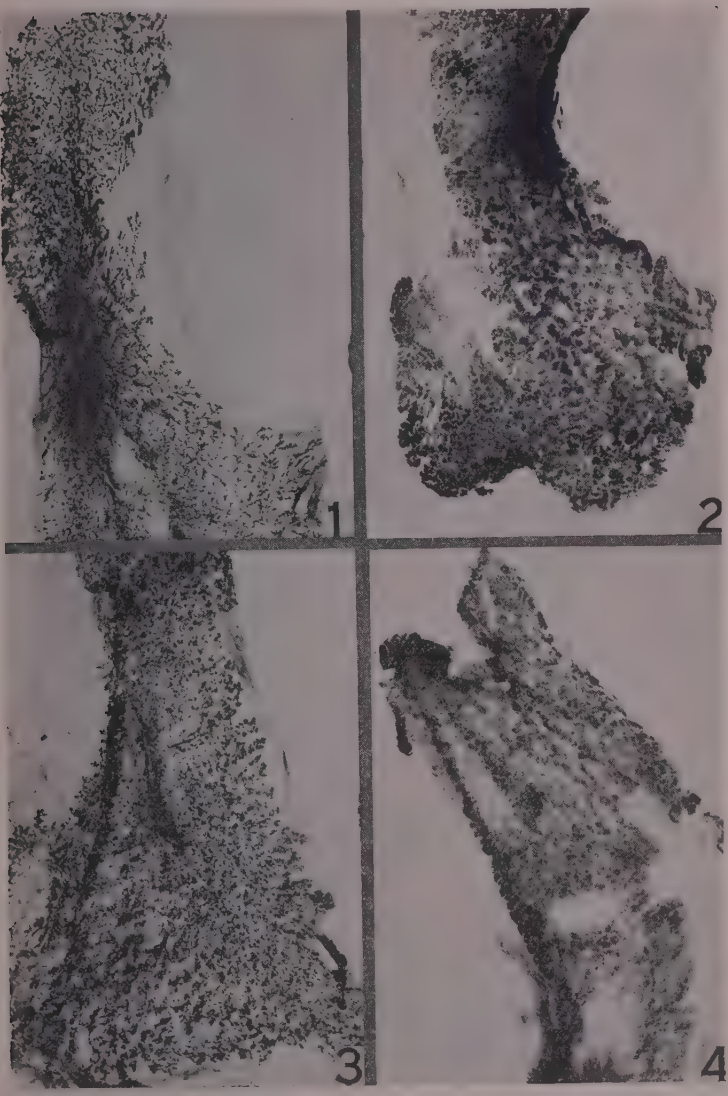
Mammary gland from a rat hypophysectomized 1 day antepartum and sacrificed on the 11th day postpartum. Alveoli have regressed to the small bud stage. No milk.

FIG. 2.

Same from a normal rat 11th day postpartum. This rat was suckling a normal litter, and therefore some of the lobules have been partially drained, while others are filled with milk.

FIG. 3.

Same from a rat hypophysectomized 1 day antepartum and injected for 10 days with 2,500 units mammotropin and 10 units adrenocorticotropin. Sacrificed on the 11th day postpartum. Some of the lobules have been maintained in a func-



tional state and contain milk, but due to insufficient dosage considerable regression has occurred.

FIG. 4.

Same from a rat hypophysectomized 1 day antepartum and injected for 10 days with 10,000 units mammotropin and 40 units adrenocorticotropin. Microscopically, as well as grossly, this gland could not be distinguished from a normal lactating gland. Its lobules appear more engorged with milk than those in Fig. 2.

All figures × 1½. Glands stained *in toto* with alum carmine, and cleared with methyl salicylate.

sufficient milk and died after 5 days. The mothers were sacrificed on day 11. Fig. 3 shows a mammary gland representative of this group. The alveolar units show considerably better development than is found in the rapidly regressing glands of the hypophysectomized control animals (Fig. 1). They were, however, less well developed than those of normal 10-days postpartum animals (Fig. 2).

Two rats received 1.0 gm. (5,000 macro-units mammotropin and 20 units adrenocorticotropin) over a period of 5 days. The mammary glands were well developed and lactating, but the young, though nursed, were not sustained. All died of inanition by day 5.

The remaining 4 rats received 2.0 gm. (10,000 macro-units of mammotropin and 40 units of adrenocorticotropin) over a 10-day period. Milk could be expressed from the nipples throughout the injection period, and was observed through the abdominal wall in the stomachs of the young. The mothers exhibited normal nesting and nursing behavior, and the young were almost continuously attached to the greatly elongated nipples. But even in this group lactation was deficient, and the original litters died by day 5. Three 5-day-old foster young from normal mothers were then given to each of these mothers. Although they were successful in obtaining milk from their foster mothers for the remaining 5 days of the experiment, they also gradually lost weight. The mammary glands of these mothers appeared grossly (Fig. 4) and microscopically like those of the normal lactating controls.

The thyroids and ovaries of the injected and control hypophysectomized rats showed regression. The thymus glands of the injected, but not of the control hypophysectomized, rats had atrophied to about one-tenth the normal weight, as a result, apparently, of adrenal stimulation. The adrenals of the injected hypophysectomized rats were not only maintained by the adrenocorticotropin, but were hypertrophic (in one case the 2 adrenals weighed 357 mg.). It is not likely, therefore, that an adrenal deficiency was the limiting factor in this experiment.

Summary. It was possible to induce postpartum lactation in hypophysectomized rats, and to maintain a functional development of their mammary glands by injections of combined mammotropic and adrenocorticotropic hormones. That this treament did not constitute complete substitution therapy was shown, however, by the fact that sufficient milk was not available for sustenance of the young.

9866

Cyclic Inhibitory Influence of the Rat's Ovary on the Uterine Response to Estrin.

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From the Department of Metabolism and Endocrinology, Michael Reese Hospital, Chicago, Illinois.*

We have recently demonstrated the participation of ovarian factors other than estrin and progestin in the phenomenon of estrus in the rat.[1] One of these ovarian factors or hormones inhibits the uterine response to administered estrogenic substance, and accounts for the marked difference in the effect of such treatment on the uteri of normal and castrated rats respectively.

The present report offers: (a) Further evidence for the existence of an ovarian factor inhibiting uterine-estrus, (b) evidence that the inhibiting factor normally operates in a cyclic manner.

(a) The Inhibitory Factor. *Methods and Results.* Each of 16 adult female rats was injected with 250 international units of dihydroxyestrin benzoate per day, for 3 days. On the fourth day the animals were anesthetized and their abdomens opened. In 11 of the animals the uterus was in diestrus, in 2 there was evidence of early estrus, and in only 3 was there definite estrus. This distribution of results is similar to that found upon opening a corresponding group of normal, untreated adult female rats. We have previously shown that the administration of estrin for as long as 20 days shows a similar lack of effect.[1] After recording the state of the uteri, 10 of the animals were ovariectomized and the abdomens of all were sutured. Three days later, without any further treatment, all the animals were sacrificed for examination. In every one of the 10 castrated animals, the uterus had developed a typical estrus reaction. The uteri in the 6 control animals were essentially unchanged. It should be noted that in all the above animals, both before and after castration, the administration of estrin resulted in a heavy cornification of the vaginal epithelium.

Comment. It is evident that ovariectomy removed an inhibitory influence which, in the normal animals, prevented most of the uteri from responding to the administered estrin, although the

* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

1 Freed, S. C., Garvin, T., and Soskin, Samuel, Proc. Soc. Exp. Biol. and Med., 1936, **35**, 409.

vaginal response was not inhibited. However, since in a few of the normal animals the uteri did respond to the estrin, it occurred to us that the stage of the estrus cycle at which the animals were injected might account for this variation in results.

(b) THE CYCLIC ACTIVITY OF THE INHIBITORY FACTOR. *Methods and Results.* Thirty-two adult female rats of the Long-Evans strain, which were showing regular estrus cycles, were selected and divided into 3 groups. Each animal of the first group was injected with 25 international units of dihydroxyestrin benzoate on the first day of its estrus cycle. This dose was repeated on the second and third day, and each animal was opened for examination on the fourth day. The 2 remaining groups of rats were similarly treated, except that the estrin injections were begun on the third and fifth days of the estrus cycle respectively.

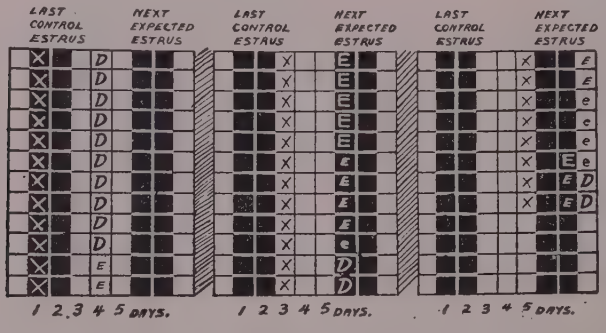

FIG. 1.

The influence of the time in the estrus cycle at which estrin is administered, upon the uterine response. The 3 blocks of results separated by the cross-hatched areas represent 3 different sets of experiments. Each horizontal row of squares in these blocks represents an experiment on an individual rat, each square being equivalent to one day of the estrus cycle. The last control estrus phase, and the next expected estrus, based on previous observation of a number of cycles, are shown in black.

X = day upon which administration of estrin was begun.
D = finding of a diestrous uterus.
e = " " " slight estrous uterus.
E = " " " moderate estrous uterus.
E = " " " full estrous uterus.

The results are graphically shown in Fig. 1. It may be seen that, with few exceptions, the state of the uteri depended upon the day of the estrus cycle upon which they were examined. The animals of the first group (whose day of examination fell on the fourth day of the cycle, *i. e.*, during the period of diestrus) exhibited the characteristic diestrus uterus. Microscoppic examination showed the state of the uterine endometrium to be consistent with the gross picture, although the vaginal epithelium in the same animals was

heavily cornified. Most of the animals in the second group (whose day of examination fell on the first day of the next anticipated estrus) showed full-blown to moderate uterine estrus, with hyperemia and fluid distension. In the third group (whose day of examination fell on the third day of the cycle) the uteri were only slightly affected, and showed some hyperemia but no distension. The animals in this last group were in the terminal stages of estrus, as evidenced by the fact that several of them which were subjected to a preliminary laparotomy on the day before their routine examination, had shown much more pronounced changes.

Comment. These results clearly show that the administration of estrin to the normal rat causes little disturbance in its uterine estrus cycle, although the vagina is markedly affected. The state in which the uterus is found depends largely upon the day of the estrus cycle upon which it is examined. This might be interpreted to mean that administered estrin never has any significant effect on the uterus of the normal rat. However, there is no reason to believe that the estrin which we injected lacks the essential physiological properties of the estrin secreted by the rats' own ovaries. And, since the results during diestrus were not entirely negative, and in view of the definite though mild effects observed in early diestrus, it seems more logical to conclude that the uterus is resistant to any estrin during its diestrus phase, but responds during estrus.

Discussion and Summary. We have demonstrated the existence of an ovarian factor in the rat, which inhibits the uterine response to estrin, and which operates in a cyclic manner corresponding to the estrus cycle of the animal. Although the development of uterine inhibition during the cycle is paralleled by the development of corpora lutea in the ovary, we do not believe that the inhibition is due to progestin. This conclusion is based on evidence that progestin, in amounts sufficient to inhibit the effects of estrin on the uterus, always results in progestational phenomena in this organ.[2, 3, 4] But, at no time in the present or previous[1] work did we observe progestational changes in the uterus. Furthermore, the antagonism between progestin and estrin also includes the inhibition of vaginal cornification,[2, 3] while in our work the vaginal mucosa never failed to become cornified after estrin administration.

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[2] Selye, H., Browne, J. S. L., and Collip, J. B., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 198.

[3] Hisaw, F. L., Meyer, R. K., and Weichert, C. A., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 754.

[4] Leonard, S. L., Hisaw, F. L., and Fevold, H. L., *Am. J. Physiol.*, 1932, **100**, 111.

To speculate briefly on the purpose served by this inhibitory factor, it may be pointed out that the estrus cycle of the rat is so short that the closely repeated stimulations of the uterus by estrin might lead to a chronic state of estrus, were it not for the presence of a mechanism which sharply limits the response of the uterus to this hormone, at the appropriate time in the cycle.

9867

Activity of Insulin in Diabetic Hyperglycemic Animals.

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Hyperglycemia is a constant accompaniment of severe untreated diabetes mellitus. Does it of itself interfere with the ability of the organism to utilize carbohydrate? Previous to the use of insulin, a fall in the blood sugar and a decrease in the severity of the diabetes was noted with fasting.[1, 2] With the use of insulin the control of hyperglycemia is accompanied by a decrease in the daily insulin requirement of patients.[3] In view of the fact that in both of these conditions the prevailing blood sugar level is lowered, the following question naturally presents itself: Does the hyperglycemia *per se* interfere with the ordinary action of insulin?

The activity of insulin at normal blood sugar levels was studied by Greeley.[4] He found that with a certain hourly intravenous dose of insulin, the blood sugar of a diabetic animal could be maintained at a normal level without change. For the individual fasting, depancreatized dog, this dose was remarkably constant over a long period of time and was consequently termed the "Basal Insulin Requirement" and expressed in units of insulin per kilogram of body weight per hour. In the present work this "Basal Insulin Requirement" was first determined on the experimental dog (at a normal blood sugar level). Then on other experimental days the animals was given insulin at this rate (or less) when the blood sugar was at a hyperglycemic level. The essential difference in procedures is

1 Allen, F. M., *J. Am. Med. Assn.*, 1914, **63**, 939.

2 Newburgh, L. H., and Marsh, P. L., *Arch. Int. Med.*, 1920, **26**, 647; 1921, **27**, 699.

3 Joslin, E. P., *Treatment of Diabetes*, Ed. 6, 305, Lea and Febiger, Phil., 1937.

4 Greeley, P. O., *Am. J. Physiol.*, 1937, **120**, 345.

that in determining the basal requirement the blood sugar level is first brought to normal levels by means of extra insulin.

Experiments were carried out on 8 depancreatized dogs which had been maintained with insulin* on a constant diet until they had recovered from the operation. Prior to the experimental periods the animals were fasted at least 24 hours and the insulin withheld 12 to 24 hours. The basal insulin requirement of each animal was determined during control periods. At other periods and when the animals were hyperglycemic, insulin was given intravenously at hourly intervals in amounts ranging from 42 to 100% of the basal requirement and the effect on the blood sugar observed. The results are reported in Table I. In 2 of these experiments the presence of sugar in the urine was determined at times when the blood sugar had ceased to change.

TABLE I.

Records of blood sugars on individual dogs for periods during which insulin was given at the hourly rates indicated at the top of the columns opposite the heading "Sub-basal Dose." The figures opposite "Basal Dose" indicate the amount of insulin that has to be given per hour to maintain the blood sugar at a constant normal level (around 100 mg.%); this determination was made during control experimental runs.

Dog	Br	Bi	M	W	J	Th	E	K
Sex	Female	Female	Male	Male	Male	Female	Female	Male
Weight, kg.	7.7	6.5	6.8	6.5	7.1	6.1	14.8	7.3
Basal dose, u./kg./hr.	.019	.023	.061	.038	.01	.037	.041	.01
Sub-basal dose, u./kg./hr.	.008	.020	.029	.020	.01	.016	.027	.01
% basal dose	42	87	47	53	100	43	65	100
Hours			Blood Sugar mg.%.					
0	320	364	384	400	276	286	482	440
1	254	—	332	284	284	276	400	—
2	222	—	—	266	250	222	—	382
3	200	335	346	—	264	206	380	—
4	190	—	336‡	276	240	186	300	286
5	182	284	312	242	250	178	346	—
6	166	—	320	266	228	182	328	206
7	166	—	286	260	234	178	306	196
8	190*	196	258§	276	224	—	298	185
9	198	—	236	266	234	180	266	182
10	182	163	222§	263	222	176	258	186
11	184	—	—	258	228	178	243	—
12	174†	—	—	265	216	174	242	—
13	174	167	—	—	—	—	—	—
14	—	168	—	—	—	—	—	—
15	—	165	—	—	—	—	—	—

*Catheterized and bladder washed.
†Catheterized: Urine sugar: trace.
‡Urinated: Urine discarded.
§Urine collected: Urine sugar: trace.

* Appreciation is expressed for a grant of Insulin (Iletin) by Eli Lilly and Co.

Additional experiments were carried out on Dog Br in which different sub-basal doses of insulin were given at hyperglycemic levels. Fig. 1 shows the control period, that is the maintenance of the normal blood sugar level by 0.020 u./kg./hr. after the blood sugar level had been brought to a normal level by higher doses of 0.040 u./kg./hr. The administration of 0.004 u./kg./hr. (26% of basal requirement) resulted in a fall from 320 to 218 mg. % at the seventh hour, after which the level remained between 182 and 230 mg. % until the thirteenth hour. At another time (not shown in the chart) 0.013 u./kg./hr. (68% of basal insulin requirement) was given and the blood sugar fell from 348 to 202 mg. % at the fourth hour, remaining between 182 and 210 mg. % until the tenth hour.

During an experiment on Dog M, 0.059 u./kg./hr. (97% of basal requirement) was accompanied by a drop from 408 to 181 mg. % at the fourth hour. Then insulin was given in amounts over the basal requirement (fifth hour: 0.090 u./kg./hr., 148%); and at the sixth and seventh hours: 0.118 u./kg./hr. (177%). At the eighth hour the blood sugar was 118 mg. % and the hourly dose of 0.059 u./kg. was again administered, the blood sugar rising to 125 mg. % at the eleventh hour.

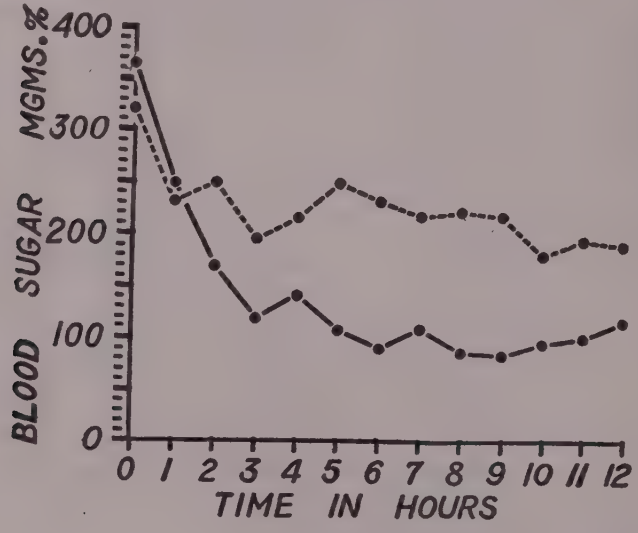

FIG. 1.

Records of blood sugars on the same animal in two separate experiments. Exp. 1 (Continuous line) blood sugar brought to normal region (120 mg.%) by injection of 0.4 u./kg./hr. insulin during the first two hours, thereafter the level is maintained by the injection of 0.02 u./kg./hr. insulin. Exp. 2 (Broken line) blood sugar course with hourly injections of 0.004 u./kg./hr. insulin.

In all cases there was a fall in the blood sugar level when insulin was injected into hyperglycemic animals at sub-basal rates. The blood sugar rose from normal levels if these amounts were given during the control periods. The rapidity of decrease in hyperglycemia varied in different animals and was not related to the percentage of the basal insulin requirement given. After a period of 2 to 11 hours, the levels remained constant for the remainder of the experimental periods. The blood sugar concentration at which these constant levels appeared, varied from 163 to 276 mg. %, and in experiments on the Dog Br did not appear to be closely related to the different percentages of the basal requirement which were given. An explanation of the fall in the blood sugar levels with sub-basal hourly doses of insulin is that kidney excretion disposes of that portion of new sugar formation of the organism which is not utilized with insulin. Variation in the rapidity of the fall results not only from differences in the amounts of sugar utilized or produced in the presence of insulin, but the actual speed with which the kidney can excrete glucose in the urine. As the falling blood sugar level approaches the kidney threshold for glucose, the amount of sugar excreted in the urine tends to equal the quantity of unutilized sugar, so that the blood sugar level ceases to change. That the activity of insulin is at least as great at these constant levels as at normal levels is shown by the results in experiments where the urinary sugar was determined and only a trace was found. Indeed it would seem that the insulin might have a hyperactivity at higher blood sugar levels.

Conclusions. 1. In 8 diabetic dogs there was a definite decrease in the hyperglycemia during the hourly injection of amounts of insulin less than that required for the maintenance of a constant blood sugar level within the normal physiological range. Hourly amounts of insulin which would have allowed the blood sugar to rise when given at a level of 100 mg. % caused the blood sugar to fall when given at hyperglycemic levels. 2. In untreated diabetes mellitus, factors other than the hyperglycemia must be responsible for the severity of the condition, as the high blood sugar *per se* does not interfere with the ordinary action of insulin.

9868 P

Lessened Incidence of Caries when Casein Replaces Milk in the Coarse Corn Meal Diet.

C. A. Lilly. (Introduced by L. H. Newburgh.)

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Hoppert, Webber and Canniff[1] reported a diet which produced dental caries in rats. This diet consisted of coarsely ground yellow corn meal 60%, whole milk powder 30%, linseed meal 6%, alfalfa meal and NaCl 1%. Although these investigators considered this diet "adequate" in every particular, they found that from 90 to 100% of their rats developed dental caries in 16 weeks (112 days).

Dental pathologists do not regard this caries as merely "fracture caries," and no satisfactory explanation of its cause has been reported. This lack of explanation constitutes a challenge to every theory regarding the cause of dental caries today.

In our studies we have found that 60% of 200 carefully controlled rats developed dental caries in 100 days when fed the Hoppert, Webber and Canniff diet. In an attempt to gain further information regarding the cause of the caries produced by this diet, the following experiment was performed.

Fifty-six rats, 30 days old, from our own inbred stock, were fed the above diet modified by substituting commercial casein for the whole milk powder. Tap water *ad lib* was allowed, and the feeding was continued for 100 days. The animals were then sacrificed and examined under light and magnification for dental caries. One large and 5 small carious lesions were found (10⅔%).

The marked reduction of the incidence of dental caries which was observed when commercial casein was substituted for powdered whole milk seems to indicate that casein exerts an inhibitory action on the production of experimental dental caries in rats which are fed a coarse corn meal diet.

Whether this inhibitory action is due to the smaller amount of lactose, the relatively low alkaline reaction, or the relatively high protein of the casein diet is a question which can be answered only by subsequent investigation.

[1] Hoppert, C. A., Webber, P. A., and Canniff, T. L., *Science*, 1931, **74**, 77.

9869

Invalidation of Plasma Ascorbic Acid Values by Use of Potassium Cyanide.

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Farmer and Abt[1] proposed a method for the determination of reduced ascorbic acid in blood plasma deproteinized by tungstic acid, which was titrated with 2,6-dichlorophenolindophenol. The method required 5 cc. of blood, "which is centrifuged immediately, the plasma removed, and a tungstic acid filtrate prepared." This quotation is given to emphasize the fact that the authors were aware of the rapid destruction of ascorbic acid in blood.[2] When the micro-method[3] requiring but 0.5 cc. of blood was developed, we recommended the substitution of metaphosphoric acid for tungstic acid as a deproteinizing agent. The dependence of plasma level upon the vitamin C intake was shown. Abt, Farmer and Epstein[4] applied these methods to the establishment of plasma values for normal individuals of various age groups as well as the variations encountered in clinical material.

In attempting to apply the macro-method, modified to the extent of substituting 10% for 5% metaphosphoric acid as a deproteinizing agent, Pijoan, Townsend and Wilson[5] report varying and inconsistent results in presumably normal individuals. They apparently overlooked the statement of Farmer and Abt as to the necessity of immediate analysis of the freshly drawn blood, and report losses in blood plasma permitted to stand for periods of 2 to 3 hours, at 26°C. and at 0° to 5°C.

In order to prevent this destruction of plasma ascorbic acid, Pijoan and Klemperer[6] and Pijoan and Eddy[7] recommended the

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1 Farmer, C. J., and Abt, A. F., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1625.

2 Contrary to the statement of Schultz, M. P.: Discussion of paper of Rinehart, J. F., Greenberg, L. D., Baker, F., and Choy, F., *J. Am. Med. Assn.*, 1937, **109**, 1394.

3 Farmer, C. J., and Abt, A. F., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 146.

4 Abt, A. F., Farmer, C. J., and Epstein, I. M., *J. Pediat.*, 1936, **8**, 1.

5 Pijoan, M., Townsend, S. R., and Wilson, A., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 224.

6 Pijoan, M., and Klemperer, F., *J. Clin. Inv.*, 1937, **16**, 443.

7 Pijoan, M., and Eddy, *J. Lab. Clin. Med.*, 1937, **22**, 1227.

addition of potassium cyanide (with potassium oxalate) to the tubes used for blood collection. This is based on the observation of Euler, Myrbock, and Larsson[8] that the oxygen uptake of ascorbic acid solutions, as measured in the Barcroft-Warburg apparatus, gave "badly reproducible values" unless potassium cyanide (1 ml. 0.005 N KCN per 10 mg. ascorbic acid) was added. Euler, *et al.*, believed that the potassium cyanide precipitated traces of heavy metals present in the system, which were responsible for the erratic values. The catalytic effect of copper was confirmed by Barron, De Meio, and Klemperer,[9] and its catalysis of oxidation of ascorbic acid added to human blood serum shown in an article by Barron, Barron, and Klemperer.[10] According to one observation of the latter authors, using whole blood of the dog, the presence of red blood cells exerts no influence on the rate of oxidation.

We, therefore, welcomed the suggestion of Pijoan and associates that potassium cyanide could be used to prevent the rapid destruction of ascorbic acid in whole blood and plasma. After some experience with the method as given by Pijoan and Eddy[7] (10 mg. KCN for 10 cc. blood) and its proportionate use in the smaller samples as required for our micromethod, we found that the use of potassium cyanide gave enhanced ascorbic acid values; in fact, potassium cyanide in aqueous solutions of metaphosphoric acid decolorizes 2,6-dichlorophenolindophenol. This was overlooked by Pijoan and associates, otherwise metaphosphoric acid-potassium cyanide blanks would have been recommended as a routine titration procedure.

The recently proposed method of Mindlin and Butler,[11] employing a photoelectric colorimeter, depends upon the increased light transmission through a 2,6-dichlorophenolindophenol solution due to partial reduction by ascorbic acid. Since a photoelectric colorimeter is not available, we are not in a position to evaluate in this simple manner the reduction of the dye upon addition of cyanide, and therefore must depend upon visual perception of the endpoint as obtained by titration. In the collection of blood, Mindlin and Butler add one drop of 5% KCN (approx. 2.5 to 3 mg.) to tubes

[8] Euler, H. v., Myrbock, K., and Larsson, H., *Z. f. Physiolog. Chem.*, 1933, **217**, 1.

[9] Barron, E. S. G., DeMeio, R. H., and Klemperer, F., *J. Biol. Chem.*, 1935-36, **112**, 625.

[10] Barron, E. S., Barron, A. G., and Klemperer, F., *J. Biol. Chem.*, 1937, **116**, 563.

[11] Mindlin, R. S., and Butler, A. M., *J. Biol. Chem.*, 1938, **122**, 673.

receiving 4 to 5 cc. of blood. It is obvious that a suitable blank should be obtained, taking this factor into account.

By a study of the reaction velocity between metaphosphoric acid plasma filtrates and 2,6-dichlorophenolindophenol buffered to pH 4.1, Minton and Butler find that no significant error in evaluating ascorbic acid is introduced by reductions caused by glutathione or cysteine within one-half minute. In the usual titration procedure, the unbuffered dye is added to the metaphosphoric acid plasma filtrate, which has a reaction about pH 2, thereby eliminating interference from -SH compounds.

Experiment I. The decolorization (blue to pink) of 2,6-dichlorophenolindophenol by potassium cyanide in 2.5% metaphosphoric acid solution.

In these experiments, the dye solution had an equivalent of 0.02 mg. ascorbic acid per cc. For titration, 0.2 cc. of a 2.5% metaphosphoric acid solution containing potassium cyanide in amounts ranging from 0.25 to 5.0 mg. per cc. was used. Titrations were made using the authors' micropipette. The data are presented in Table I.

It will be seen that the decolorization is not a straight line function of the amount of cyanide in the solution being titrated. The effect is relatively greater in lower concentrations of KCN.

TABLE I.
The decolorization of 2,6-Dichlorophenolindophenol by KCN.

Period of Standing (Room Temp.)	KCN-HPO_3 Solution mg./cc.	Titre† cc. Dye for 0.2 cc. Solution cc.	Blank 0.2 cc. 2.5% HPO_3 cc.	Dye Decolorized by KCN cc.
Immediate	5	.066	.005	.061
½ hr.	5	.065	.004	.061
1 "	5	.064	.004	.060
2 "	5	.065	.004	.061
Immediate	1	.023	.005	.018
½ hr.	1	.021	.004	.017
1 "	1	.022	.005	.017
2 "	1	.021	.004	.017
Immediate	.25*	.011	.004	.007
½ hr.	.25	.011	.004	.007
1 "	.25	.010	.004	.006
2 "	.25	.011	.004	.007

*The amount of KCN in 2 cc. Plasma-HPO_3 filtrate used by Pijoan and associates is 0.4 mg. In the authors' micromethod, the amount would be 0.05 mg. in 0.2 cc. filtrate.

†The end point persists for 20 seconds. Fading occurs on longer standing, as in blood.

Experiment II. The stability of ascorbic acid in 2.5% metaphosphoric acid solution.

In this experiment, approximately 1 mg. of ascorbic acid* was dissolved in 100 cc. of 2.5% HPO_3 solution. To 50 cc. we added 12.5 mg. KCN. The data (Table II) are expressed as cc. of dye (1 cc. $\simeq$ 0.02 mg. ascorbic acid) required to titrate 0.2 cc. portions (by micropipette) after various periods of standing. The 50 cc. flasks containing the samples were lightly corked.

TABLE II.
Stability of Ascorbic Acid.

Time of Standing (Room Temp.)	Without KCN			With KCN (0.25 mg./cc.)		
	Titre	Blank 0.2 cc. 2.5% HPO_3	Dye Reduced by Ascorbic Acid (alone)	Titre	Blank 0.2 cc. 2.5% HPO_3	Dye Reduced by Ascorbic Acid (+KCN)
Immediate	.033	.004	.029	.040	.004	.036
15 min.	.032	.004	.028	.038	.004	.034
30 "	.032	.004	.028	.038	.004	.034
60 "	.030	.004	.026	.037	.004	.033
90 "	.031	.004	.027	.037	.004	.033
180 "	.030	.004	.026	.037	.004	.033

The data indicate no advantage in preservation of ascorbic acid in 2.5% metaphosphoric acid by KCN, while the enhancement of the titration is clearly shown.

Experiment III. The enhancement of apparent plasma ascorbic acid values by the use of KCN.

Twenty cc. of blood were taken from the vein of an individual. Of this, 10 cc. were placed in a tube containing 10 mg. KCN and 15 mg. potassium oxalate. The remaining 10 cc. were placed in a tube containing 15 mg. potassium oxalate alone. Five cc. of each blood were centrifuged, the plasma pipetted off, and placed in respective tubes. One cc. of each plasma was removed and deproteinized according to the method of Pijoan and Eddy,[7] then 2 cc. of the resulting plasma-HPO_3 filtrate titrated immediately (A in Table III). After 30 minutes, a second 1 cc. sample of each plasma was deproteinized and titrated in the same way (B in Table III).

The unused 5 cc. portions of each whole blood sample were allowed to stand in the laboratory for 30 minutes. They were then centrifuged, deproteinized, and titrated as above (C in Table III).

The data are given in Table III.

* We wish to express our appreciation to Merck and Co., Inc., Rahway, N. J., for a generous supply of ascorbic acid (Cebione).

TABLE III.
Ascorbic Acid Values (mg.%) from Individual Blood as Effected in KCN.

	No KCN mg.%	With KCN mg.%
A. Plasma separated immediately from blood	.616	.836
B. Plasma 30 minutes after separation from blood*	.528	.700
C. Plasma removed after blood had stood 30 min.*	.572	.616

*Room temperature.

From the figures on plasma separated immediately from blood when drawn, it is seen that the use of cyanide gives an enhancement of 0.220 mg. %, which is approximately 25% of the value of ascorbic acid present. These data are typical of 5 similar experiments.

Experiment IV. The enhancement of apparent ascorbic acid values in plasma as determined by the micromethod of Farmer and Abt, from blood containing potassium cyanide.

Blood from the same individual was collected in 2 tubes, one containing potassium oxalate, the second, oxalate with KCN in the amount of 1 mg. per cc. blood. As in the previous experiment with the macromethod, a portion of each blood was centrifuged imme-

TABLE IV.
Influence of KCN on Plasma Ascorbic Acid Values Determined by Micromethod of Farmer and Abt. (Figures are expressed as mg.%.)

Time of Standing (Room Temp.)	Plasma Separated and Deproteinized Immediately No KCN	Plasma Separated Immediately. Deproteinized at Time Interval Shown		Plasma Separated from Whole Blood at Time Interval Shown, then Immediately Deproteinized	
		No KCN	With KCN	No KCN	WithKCN
Blood A					
Immediate	1.20	1.20	1.40	1.20	1.40
½ hr.	1.20	1.12	1.36	1.16	1.32
1 "	1.16	1.00	1.16	1.08	1.16
2 "	1.08	1.00	1.08	1.04	0.96
6 "	0.76	0.60	0.60	0.68	0.56
Blood B					
Immediate	.64	.64	.80	.64	.80
¼ hr.	.64	.64	.80	.60	.76
½ "	.64	.52	.72	.56	.68
1 "	.64	.44	.68	.60	.60
2½ "	.60	.44	.64	.52	.60
Blood C					
Immediate	.36	.36	.64	.36	.64
½ hr.	.32	.28	.56	.32	.48
1 "	.28	.20	.52	.28	.40
2 "	.28	.16	.44	.20	.40
3 "	.24	.16	.44	.20	.36
5 "	.24	.12	.36	.16	.32

diately, then deproteinized in the usual way (0.1 cc. plasma + 0.1 cc. water + 0.2 cc. 5% HPO_3) and 0.2 cc. deproteinized plasma titrated with 2,6-dichlorophenolindophenol (1 = 0.02 mg. ascorbic acid) using our micropipette. A portion of each plasma was held for later titration at hourly intervals.

The remainder of each blood sample was allowed to stand at laboratory temperature and a portion of each centrifuged and deproteinized at intervals as shown in Table IV. We present data on 3 bloods of ascorbic acid contents frequently observed.

The above data are typical of similar studies conducted upon 17 different bloods.

Whole blood to which KCN is added becomes hemolysed after a short period of standing.

Bloods differ one from another considerably in the loss of reductive power upon standing.

Conclusion. For dependable ascorbic acid values, blood should be centrifuged, the plasma deproteinized, and the plasma-HPO_3 filtrate titrated in immediate sequence after the blood is drawn. Whole blood which stands in a closed small phial, with a minimum air space, may be depended upon to give results of clinical value for ½ hour. The higher values obtained with bloods to which KCN has been added represent an enhancement due to the action of KCN upon the 2,6-dichlorophenolindophenol, and in no wise a more accurate determination of their ascorbic acid content. This is particularly true with blood of low ascorbic acid value. KCN does not prevent the loss of ascorbic acid from blood.

It is a pleasure to acknowledge our indebtedness to Mrs. Jessie Maaske for technical assistance, and to Mr. Herman Chinn for checking several titrations.

9870 P

Vitamin B_1 Metabolism in Man. Excretion of B_1 in Urine and Feces.

ALFRED S. SCHULTZ, ROBERT F. LIGHT AND CHARLES N. FREY.
(Introduced by I. Greenwald.)

From the Fleischmann Laboratories, Standard Brands Incorporated, New York, N. Y.

Goodhart and Jolliffe[1] have emphasized the need for a practical chemical method for studying vitamin B_1 metabolism. The fer-

[1] Goodhart, Robert, and Jolliffe, Norman, *J. Am. Med. Assn.*, 1938, **110**, 414.

mentation method recently published by our laboratories[2, 3] is, we believe, admirably suited to this purpose.

After, and in conjunction with, considerable work on rat metabolism of vitamin B_1, we have applied our method to a group of human subjects. Five adults and 3 children, all 'normal' subjects with the possible exception of Subject E, who was under treatment for nervous breakdown, coöperated with us in this project. The main diet was in all cases normal, *i. e.*, no attempt was made to restrict these individuals to a standard diet. The average daily urinary output of the adults (average of 32 determinations) was 497.7 ± 47 gamma. For the children the urinary average was 333 ± 60 gamma.

Having established the normal urinary output on the average or normal diet, doses of 5 and 10 mg. (5000 and 10,000 gamma) were given once daily *per os* to the adult subjects. Table I gives the results obtained in this experiment.

TABLE I.
Daily Excretion of B_1 in the Urine.

B_1 dose per day	No. days on diet	Urinary B_1 of Subjects per Day				
		A	B	C	D	E*
Normal diet	8	Average for group 497 ± 47 gamma				
" + 5000 gamma	1	800	1000	1300	1320	600
" + " "	2	1000	1375	1800	1600	660
" + " "	3	1200	2000		1700	700
" + " "	4		1500			1068
" + " "	5-8 (aver.)					969
" + " "	19-21 "	1687	2383			
" + 10,000 "	Aver. maximum excreted		2414		2400	

*Not normal.

The first point to be noted is the non-uniform response to the 5 mg. dose. We feel that this variation in response may be related to either the level of body stores or individual rates of absorption.

After sufficient time has elapsed on the 5 mg. level, we find that urinary excretion of the vitamin takes place at a steady rate. This condition we designate as excretion equilibrium. When a subject in excretion equilibrium at 5 mg. per day is placed on the 10 mg. level, we do not obtain a significant rise in urinary output even after 15 days at this level. This phenomenon cannot be explained by body storage and the explanation must be sought elsewhere. Accordingly we made a series of parallel observations on the vitamin content of urine and feces. The results of this experiment are contained in Table II.

2 Schultz, A. S., Atkin, L., and Frey, C. N., *J. Am. Chem. Soc.*, 1937, **59**, 948.
3 Schultz, A. S., Atkin, L., and Frey, C. N., *J. Am. Chem. Soc.*, 1937, **59**, 2457.

TABLE II.
Daily Excretion of Vitamin B_1 in Urine and Feces.

	Aver. daily excretion after 6 to 19 days on supplement of 10,000 gamma Vit. B_1 per day		Aver. daily excretion on 2nd and 3rd days after reducing supplement from 10,000 to 5,000 gamma Vit. B_1 per day	
	Urine	Feces	Urine	Feces
Subject D	2350 gamma	7360 gamma	2600 gamma	2210 gamma
Subject F	2600 "	6900 "		

Primarily, Table II shows that doses in excess of 5 mg. result in incomplete absorption. Indeed, we find virtually all of the additional vitamin in the feces, *i. e.*, 5 mg. (5000 gamma). Thus the results clearly indicate that one must determine vitamin B_1 in feces as well as urine in order to obtain a true picture of vitamin metabolism.

It does not necessarily follow that incomplete absorption manifests itself only at doses of 5 mg. and above, for we find considerable fecal excretion of the vitamin at the 5 mg. level. Subject E. described in Table I as not normal, apparently failed to absorb vitamin B_1 in excess of 1-2 mg. per day. The indications are that if we had examined his feces when he had reached excretion equilibrium at the 5 mg. level, we would have found 2-3 mg. not being absorbed.

The oral administration of 5 to 10 mg. of the vitamin daily is considered, if we may judge from current medical literature, to be a moderate dosage. The data which we have presented would show that oral doses of this size are excessive or at least inefficient.

Schultz, Atkin and Frey[3] raised the question of the identity of the vitamin measured by the fermentation method. They showed that a fraction of the vitamin molecule 2-methyl-5-ethoxymethyl-6-aminopyrimidine will also give the fermentation response. However, irrespective of whether or not the urine and feces responses obtained represent true vitamin B_1, there is a close correlation between the determined values and the vitamin B_1 intake. The indications are that the method may be a valuable aid in the study of human vitamin metabolism.

9871

Chemotherapy of *B. pertussis* Infections of Mice.

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It has been demonstrated that sulfanilamide and other sulfur-containing compounds possess a surprising degree of versatility as chemotherapeutic agents in a variety of very dissimilar infections. The amenability of *B. pertussis* infections in mice to chemotherapy has, up to the present, not been investigated.

Kolle flasks containing Bordet-Gengou medium were heavily seeded with a broth suspension of *B. pertussis* (9077 M_2)* and grown for 68 hours. The growth from 3 flasks (about 290 sq. cm.) was suspended in 24 cc. of broth and 0.5 cc. of this suspension was injected intraäbdominally into each of 40 mice. A simultaneous titration subsequently showed this inoculum to be between 2 and 4 M.L.D.

These mice were divided into 4 groups of 10 each. One group remained untreated. The other 3 groups were treated orally one hour after the inoculation as follows: the first, with 25 mg. of sulfanilamide;† the second, with 25 mg. of 4,4′-di-(acetylamino)-diphenylsulfone;‡ and the third, with 25 mg. of 4,4′-diaminobenzenesulfonanilide.‡

Within 21 hours 80% of all animals were dead, and the remainder, in less than 45 hours. Treatment did not affect even the survival time. Titration mice infected with one-half of the inoculum were dead in less than 21 hours, while other titration animals infected with smaller doses remained alive.

Identical results were obtained in another series of 40 mice similarly infected and treated.

Conclusions. Sulfanilamide, 4,4′-di-(acetylamino)-diphenylsulfone, and 4,4′-diaminobenzenesulfonanilide have no therapeutic value in the treatment of mice infected intraäbdominally with virulent *B. pertussis*.

* Kindly supplied by Dr. Pearl Kendrick of the Michigan Department of Health.

† Kindly supplied by E. R. Squibb & Sons, New York.

‡ Synthesized and donated to us by the Monsanto Chemical Company, St. Louis, Missouri.

9872 P

Effect of the Pulse on Lymph Formation and Interstitial Movement of Substances.

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The results of recent investigations suggest that connective tissue fibers serve as pathways for the extravascular transport of vital dyes escaping from lymphatics.[1] In unpublished studies on the ear of the mouse, gentle, alternating, external pressures (0 to 2 cm. or 8 cm. of water) caused squeezing of the tissues and bending of these fibers and at the same time the rapidity of spread of vital dyes through the interstitial tissues was increased. That gentle massage increases the lymph flow from a part is well known.[2] The work to be reported now deals with the effect of pulsation of the blood vessels both upon lymph flow and upon the movement of substances through the interstitial tissues.

Warmed, defibrinated, aerated rabbit's blood was perfused for periods of 1 to 1½ hours, through freshly cannulated rabbit's ears, with the use of an apparatus providing pulsatile and non-pulsatile pressures. "Systolic" and "diastolic" pressures of 141/60 mm. of mercury were used in the case of the pulsatile pressure at about 100 "beats" per minute, while for the constant pressure the equivalent of 141 mm. of mercury was employed. It will be seen that a somewhat higher mean pressure was used in the latter instances. Lymph formation and flow were estimated by filling a few peripheral lymphatics near the tip of the ear with a vital dye and noting the movement of the dye colored lymph toward the base of the ear. The method has been previously used in human skin.[2] To study the rate of spread of substances through the tissues, minute amounts (0.01 to 0.02 cu. mm.) of a vital dye (pontamine sky blue) were introduced through micropipettes into the subepidermal connective tissue of the ear by capillarity without utilization of pressure. Camera lucida drawings of the outline of the dye spots were made immediately after placing the dye in the tissues and again half an hour and 1 hour later. To express the rate of interstitial spread of dye, the area of each spot as determined after one hour was divided by

[1] McMaster, P. D., and Parsons, R. J., Proc. Soc. Exp. Biol. and Med., 1938, **37**, 707.

[2] McMaster, P. D., *J. Exp. Med.*, 1937, **65**, 347.

its initial area. For comparative data, only dye spots of approximately the same initial area were used.

Lymph flow was 15 to 20 times more rapid in the ears perfused with pulsatile pressure, despite less blood flow per gram of ear per minute in most instances. In the pulsatile perfusion experiments, the lymph channels transported the blue dye from near the tip to the base of the ear in 7 to 12 minutes and the color began to pale in the channels in 12 to 20 minutes as the result of dilution by newformed lymph. In many instances segments of the channels had become colorless after 20 minutes. In the constant pressure perfusions, the dye reached the base of the ear in only one of 13 instances and then after 46 minutes had elapsed. Clearance of color in the channels was never observed in these.

Edema sometimes developed during the course of a pulsatile perfusion and as it did so lymph flow increased enormously even when the volume of blood perfused was relatively small. Very little lymph flow attended the edema that occurred in the constant pressure perfusions.

Perfusion with pulsatile pressure greatly increased the rate of spread of dye through the tissues. One group of data will serve to illustrate the results. The camera lucida drawings of the dye spots used had initial areas of between 100 and 125 planimetric units and no edema occurred around any of them. See Table I.

TABLE I.
Spread of Dye Spots in Interstitial Tissue.
Initial Area—100 to 125 Planimetric Units.

Ears perfused with	No. of Dye Spots	Aver. Initial Area, P.U.	Increase in Size of Dye Spots final area ÷ initial area	
			Extremes	Average
Pulsatile pressure	6	112	4.5 to 8.7	5.4
Constant pressure	7	112	3.2 to 5.0	3.8

Pulsation of the blood vessels seems to provide the motive force for lymph flow through the resting, unmoved tissue. It seems probable that much of its effect is exerted through the connective tissue fibers associated with the walls of the blood vessels and lymphatics. The extent to which other factors are responsible is now under investigation.

Physiologists have long known that perfusions at pulsatile pressure are more efficient than those at constant pressure. The more rapid movement of substances through the tissues and the more rapid formation and removal of lymph, as demonstrated in the present work, will largely explain this fact.

9873

Influence of a Pancreas Extract and Other Proteins on Liver Fat and Ketosis.

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Some years ago while carrying out experiments on the relation of the preceding diet to the extent of the fasting ketosis in albino rats we were surprised when a much greater ketonuria was observed with animals which had been for a few days on a diet composed of butter 65 and glucose 35, than one in which the glucose was replaced by casein. We now know that this was associated with the fatty livers which are produced by low protein diets.[1, 2, 3] It has been found[4] that the fat leaves these livers more quickly when the rats are fasted if they are given a pancreas extract which Dragstedt, *et al.*, have shown[5] prevents the deposition of fat in the livers of depancreatized dogs maintained on insulin and which, believing it to be a new fat metabolizing hormone, they have named *lipocaic*. We were primarily interested in the influence on ketosis of the more rapid loss of fat from the fatty livers on fasting.

In Experiment 1 the rats had been on the low protein diet[1] for 10 days before being fasted. Urine collections were made daily, nitrogen determinations carried out by the macro Kjeldahl method and total ketones determined by Van Slyke's well known method. The pancreas extract was prepared according to Dragstedt's directions.[5] The solution administered in Experiment 1 contained 48.2% total solids and 5.5% nitrogen. There was a marked diminution in the degree of ketonuria as the dose of pancreas extract was increased. A ketonuria was produced in Experiment 2 by feeding each rat 3 cc. per day of a mixture half butter fat and half cotton seed oil and in Experiment 3 by the method of Butts and Deuel[6] using sodium caprylate.[7] One and a half cc. of 5% solution was adminis-

1 Channon, H. J., and Wilkinson, H., *Biochem. J.*, 1935, **29**, 350.

2 Best, C. H., and Channon, H. J., *Biochem. J.*, 1935, **29**, 2651.

3 Beeston, A. W., Channon, H. J., and Wilkinson, H., *Biochem. J.*, 1935, **29**, 2659.

4 MacKay, E. M., *Am. J. Physiol.*, 1937, **119**, 783.

5 Dragstedt, L. R., Van Prohaska, J., and Harms, H. P., *Am. J. Physiol.*, 1936, **117**, 175.

6 Butts, J. S., and Deuel, H. J., Jr., *J. Biol. Chem.*, 1933, **100**, 415.

7 Butts, J. S., Cutler, C. H., Hallman, L., and Deuel, H. J., Jr., *J. Biol. Chem.*, 1935, **109**, 597.

tered to each rat by stomach tube twice daily. In both experiments the ketosis was reduced by the pancreas extract containing in this case 31.5% solids and 3.8% nitrogen. We have found that other factors which rapidly reduce a high liver fat during fasting[8] also reduce the degree of ketosis as measured by the ketonuria.[9] Deuel, *et al.*,[10] have found that although choline causes the fat to leave the liver rapidly it does not reduce the ketosis very much.

There is some question as to whether or not Dragstedt's preparation[5] contains a new lipotropic substance. Presumably he ruled out the lecithin or choline content as being responsible.[11] Unfortunately histological instead of chemical methods were used for estimating liver fat. In comparing raw or autoclaved pancreas with choline equivalent to that which it contained in its influence upon the liver fat when raised by a low protein diet Aylward and Holt[12] came to the conclusion that the effect was similar. An examination of their data shows that the amount of liver fat in relation to body size is usually considerably greater when choline was given than after an equivalent amount of pancreas. However, Best[13] has found that the lipotropic effect of Dragstedt's *lipocaic*[5] in rats with fatty livers can be accounted for by the choline plus the protein content. The experiments in Table II have a bearing upon this point. Liver "fat" was determined by saponification, acidification and extraction with petroleum ether. It therefore represents the total fatty acids plus nonsaponifiable lipids. The pancreas extract (Exp. 6) was much more potent than either casein or the crude proteins of skeletal muscle (beef) in preventing the deposition of fat in the liver. When included in the diet in sufficient quantity the pancreas extract caused the liver fat to remain approximately normal in amount (Exp. 7).

In Experiment 8 a rough separation of the protein and possible choline content of the pancreas extract was made. Five hundred and fifty grams of the pancreas extract used in Experiment 1 was added to water with a final volume of 1100 cc. This is noted on the last column in Table II as pancreas extract A and the solution contained 24.1% total solids and 2.75% nitrogen. Eight hundred cc.

[8] MacKay, E. M., *Am. J. Physiol.*, 1937, **120**, 361.

[9] MacKay, E. M., and Barnes, R. H., *Am. J. Physiol.*, 1937, **118**, 184.

[10] Deuel, H. J., Jr., Murray, S., Hallman, L. F., and Tyler, D. B., *J. Biol. Chem.*, 1937, **120**, 277.

[11] Van Prohaska, J., Dragstedt, L. R., and Harms, H. P., *Am. J. Physiol.*, 1936, **117**, 166.

[12] Aylward, F. X., and Holt, L. E., Jr., *J. Biol. Chem.*, 1937, **121**, 61.

[13] Best, C. H., personal communication.

TABLE I.

Experiments 1, 2 and 4—averages of groups of 4 females each.
Experiments 3 and 5—averages of groups of 4 males each.

Exp.	Group	Body Wt., gm.	Body Surface, sq.cm.	Urine ketones in mg. per sq. dcm. B.S. per day					Urine nitrogen in mg. per sq. dcm. B.S. per day					Dose per 100 sq. dcm. Body Surface per day		
				1	2	3	4	5	1	2	3	4	5	*Pancreas Extract mg.	Nitrogen mg.	Casein mg.
1	1	144	311	2	26	15			18	21	14			0	0	
	2	141	306	2	15	19			21	17	16			32	4	
	3	139	304	2	14	16			14	17	14			46	5	
	4	139	304	2	15	11			23	19	20			80	9	
	5	144	311	1	0	3			26	29	27			158	18	
	6	139	304	0	0	1			30	32	35			316	36	
2	1	186	370	2	5	5	2	3	23	19	21	14	14	0	0	
	2	191	377	1	1	0	0	1	20	35	27	23	25	195	24	
3	1	262	464	2	8	11	27	10	39	25	25	21	23	0	0	
	2	261	463	3	3	1	4	1	37	35	28	27	30	159	19	
4	1	181	363	1	8	15	14	12								0
	2	172	351	2	10	16	12	9								25
	3	171	349	1	15	10	4	8								50
	4	171	349	0	1	0	0	0								100
5	1	250	450	5	16	10	8	12								0
	2	249	449	1	12	8	6	4								25
	3	251	451	2	10	2	4	6								50
	4	252	452	0	1	0	1	0								100

*Calculated here on a dry basis but actually administered in solution.

TABLE II.

Averages for 6 female rats in each group. Initial weight when removed from stock diet. Final weight after 12 days on the low protein, high fat diet. Except Exp. 6 which had 4 male rats in each group and were on the diet 7 days.

Exp.	Group	Body Wt. Initial gm.	Body Wt. Final gm.	‡Body Surface sq.cm.	Liver Wt. Actual gm.	Liver Wt. Mg. per sq. dcm. Body Surf.	Liver Fat %	Liver Fat mg. per sq. dcm. Body Surf.	†Food Intake in grams per sq. dcm. B.S. per day *Special diet	Pancreas Extract	Casein	Skeletal Muscle	Pancreas Extract cc. per sq. dcm. B.S. per day
6	1	177	187	371	7.42	2000	8.2	164	2.29				
	2	167	180	362	6.49	1795	4.1	74	2.05	.18			
	3	173	173	352	6.42	1825	5.6	102	1.80	.08			
	4	173	173	352	6.79	1935	5.9	114	2.15	.04			
	5	175	181	363	6.55	1805	6.9	125	2.37	.02			
	6	167	175	355	6.80	1920	6.6	127	1.97		.17		
	7	184	191	377	7.33	1945	7.3	142	1.78		.07		
	8	171	180	362	7.83	2165	8.2	178	2.22		.04		
	9	180	180	362	6.86	1900	8.9	169	2.18		.02		
	10	180	175	355	6.74	1900	7.8	148	2.15			.19	
	11	180	185	369	6.93	1880	8.4	158	2.32			.10	
	12	180	185	369	7.01	1900	8.1	154	2.30			.05	
	13	177	173	352	6.75	1930	8.8	170	2.26			.02	
7	1	157	138	304	5.84	1920	14.7	282	1.97				
	2	144	143	309	5.86	1865	4.1	76	2.00	.22			
	3	152	144	311	5.95	1915	3.9	75	1.56	.34			
	4	144	150	321	6.86	2135	7.6	162	1.80	.16			
	5	142	151	322	6.27	1945	6.3	123	1.51	.30			
8	1	139	135	299	5.15	1720	6.7	115	2.66				0
	2	138	136	301	4.92	1635	7.4	121					0
	3	137	142	308	5.52	1785	3.1	55	2.23				1A
	4	139	142	308	6.07	1970	3.5	69					2A
	5	148	157	330	5.80	1755	3.3	58	2.29				1B
	6	139	150	321	6.15	1920	3.2	61					2B
	7	139	142	308	5.50	1780	3.5	62	2.05				1C
	8	141	146	314	5.38	1710	3.7	63					2C

*The same low protein, high fat diet as used in other experiments, butter fat 40, glucose 45, brewers yeast 5, vitamin free casein 5, and Osborne and Mendel's salt mixture 5.

†For 12-day period on the special diet, with and without additions. The body surface used was calculated from the average body weight (Carman, G. G., and Mitchell, H. H., *Am. J. Physiol.*, 1928, **76**, 381) of the 12-day period. The pancreas extract is calculated on a water-free basis, the casein was vitamin free and the skeletal muscle was dried powdered lean beef.

‡Body surface calculated on final weight for liver figures.

of this were poured into 16 liters of 95% ethyl alcohol with vigorous stirring and a white colloidal precipitate was formed. This settled out over night forming a brown paste with a deep yellow supernatant liquid above which was filtered off. The paste, free of practically all the choline it might contain but which represents the major portion of the protein in the pancreas extract, was dried so that it was free of alcohol and water added to a final volume of 400 cc. which was pancreas extract B. It contained 40.2% solids and 4.01% nitrogen. The supernatant liquid was evaporated to dryness and the residue taken up in water so that the final volume was 400 cc. making extract C. It would contain the bulk of any choline in the extract. The solids and nitrogen content were 7.0% and 1.51% respectively. The two fractions (extracts B and C) were about equally effective in preventing the accumulation of fat in the liver. This result supports Best's contention[18] that both the protein and choline contained in the pancreas extract act to prevent the deposition of fat in the liver of the rat when on a diet low in protein and choline but high in fat.

Since choline does not appreciably affect the ketosis of fasting fatty liver rats[10] it was desirable to compare an equivalent amount of protein with the pancreas extract in its antiketogenic effect. Casein was selected for this purpose and in Experiment 4 can be seen to be equally as effective as the same amount of pancreas extract (both on a dry weight basis) in antiketogenic activity. In this experiment the ketosis was produced in the same manner as in Experiment 1, the rats having been on the fatty liver producing diet for 10 days before fasting was commenced.

Summary. Although they do not offer direct proof our experiments support the view that the reduction in liver fat caused by the pancreas extract which Dragstedt has described as *lipocaic* is due to the sum of the lipotropic effects of the choline and protein which it contains. The influence of the pancreas extract upon the fat content of the livers of rats on a low protein and low choline but high fat diet is greater than that of a similar amount of protein in other forms. The ketosis which results when rats with such fatty livers are fasting is reduced about equally by the pancreas extract and an equivalent amount of casein. Althought both the choline and the protein contained in the pancreas extract affect the fat content of the liver, choline is without antiketogenic activity under the conditions studied here.

9874

Blood Vessel Anastomosis with Payr Cannulæ and without Anti-Coagulant, in Acute Experiments.

C. Heymans.

From the Department of Pharmacology, University of Ghent, Belgium.

Kabat recently stated[1] that, "arterial anastomosis by means of Payr cannulæ, the method recommended by Heymans and his co-workers,[2] proved unsatisfactory in our laboratory." Inasmuch as we have been using this method, without anticoagulant, entirely satisfactorily for the past 15 years for such varied cross-circulation experiments as perfusion of the isolated head or brain, isolated carotid sinus and perfusion of acutely transplanted spleen, kidney, leg, intestine, carotid sinus and suprarenal, we wish to describe in detail our procedure in blood vessel anastomosis so that this technique may be successfully applied by other workers.

The Payr cannulæ, made of thin brass tubes with 2 external grooves (Fig. 1, A), vary in size from 2 to 8 mm. in diameter,

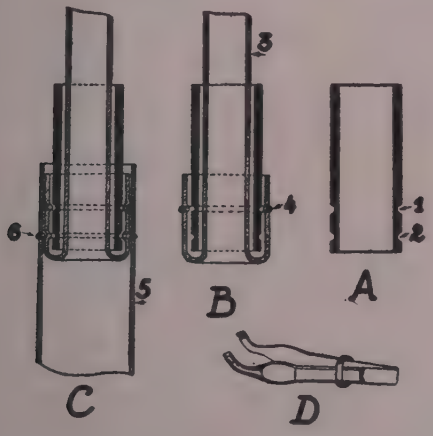

Fig. 1.

A, shows the appearance of the cannula in sagittal section and indicates the position of the grooves (1, 2).

B, shows the position of the first ligature (4) on the everted vessel (3).

C, shows the second vessel (5) drawn over the first and the position of the second ligature (6).

D, shows the cannula holder. Note the grooved prongs of this holder.

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[1] Kabat, Herman, Proc. Soc. Exp. Biol. and Med., 1938, **37**, 698.

[2] Heymans, C., Bouckaert, J. J., and Regniers, P., *Le sinus carotidien et la zone homologue cardio-aortique*, G. Doin et Cie, Paris, 1933.

and from 8 to 16 mm. in length. The cannula, corresponding in diameter to the size of the smaller of the 2 blood vessels to be connected (be they arteries or veins) is selected and fixed in the special cannula holder (Fig. 1, D). The ligated vessel is pulled through the cannula and a "bull dog" clip is placed below the ligature. The vessel is then cleanly cut between the ligature and the clip and the severed edge is carefully grasped by 3 delicate ocular forceps and everted over the edge of the cannula (Fig. 2, A). As soon as the vessel has been pulled down far enough over the cannula one forceps is removed from the edge and is placed about the vessel and cannula to hold it for tying. It is then firmly tied in the second groove (Fig. 1, B, 4).

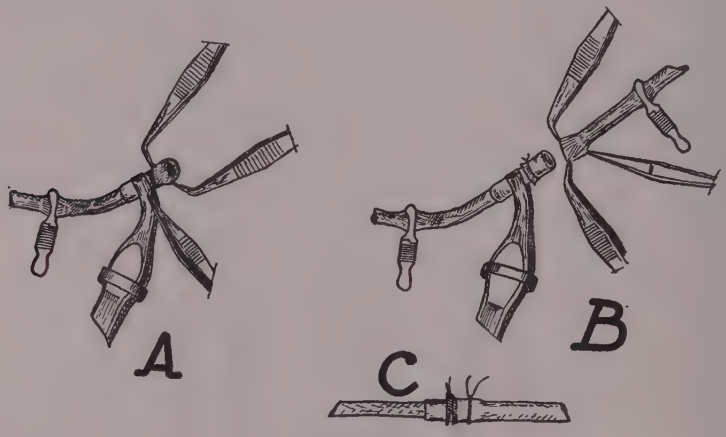

Fig. 2.

Shows, in A, the method of grasping and everting the first vessel over the cannula with the cannula holder in place. B, shows the method of slipping the second vessel over the first, everted one. C, shows the completed anastomosis from the outside.

The inside of the vessel, so secured, is washed with physiological saline to the level of the "bull dog" clip, care being taken not to injure the intima. The everted intimal surface is also carefully washed. For this procedure a round, blunt, hypodermic needle on a syringe has been found very useful.

The vessel to be connected is ligated and divided. A short distance below the ligated end to be connected, a "bull dog" clip is placed, as in the first vessel, and the vessel is cleanly severed between the ligature and the clip. The severed edges are grasped by the 3 ocular forceps. At this time the lumen of this vessel, as far as the level of the "bull dog" clip, is washed with physiological saline, as before, and the vessel is pulled over the previously tied

and everted vessel (Fig. 2, B). As soon as the vessel has been pulled down far enough, one forceps is removed from the edge and is placed about the vessel and cannula to hold it for tying. The outer vessel is tied in the first groove, at 6, Fig. 1, C. The "bull dog" clips may now be removed from both vessels.

The anastomosis, thus completed, approximates endothelium to endothelium without the possibility of metal or thread coming into contact with the blood flowing through the anastomosis (Figs. 1, C and 2, C).

This method of blood vessel anastomosis is very simple, can be performed in a few minutes and is always successful if properly done. Neither thrombosis nor leakage occur after many hours of blood flow. It should also be pointed out that with the above described technique not only can anatomically similar vessels of quite different calibre be anastomosed together, be they arteries or veins, but artery may be anastomosed to vein, or *vice versa*.

9875

Conversion of Succinic Acid to Glucose in the Phloridzinized Dog.

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Koranyi and Szent-Györgyi have reported[1] that succinic acid will decrease the ketosis in diabetes. Although other investigators[2, 3] have been unable to confirm this observation it has renewed interest in the behavior of succinic acid in metabolism. Ringer, Frankel and Jonas in a widely quoted study[4] found that extra glucose was excreted when sodium succinate was fed to the phloridzinized dog. It seemed desirable to reëxamine this point for various reasons. Our experiments are summarized in Table I. All pertinent data are included with the exception that 6 gm. of NaCl were administered daily by stomach tube in order to obtain good urine volumes. The bladder was emptied by catheter at the end of each 24-hour period of urine collection. Urine ketones were determined

1 Koranyi, A., and Szent-Györgyi, A. V., *Dtsch. med. Wschr.*, 1937, **63**, 1029.
2 Lawrence, R. D., McCance, R. A., and Archer, N., *Brit. Med. J.*, 1937, **2**, 214.
3 Dunlop, D. M., and Arnott, W. M., *Lancet*, 1937, **233**, 738.
4 Ringer, A. I., Frankel, E. M., and Jonas, L., *J. Biol. Chem.*, 1913, **14**, 539.

TABLE I.

Day	Urine Excretion: Urine Vol. ml.	Total acetone bodies gm.	Nitrogen gm.	Dextrose gm.	D:N	Extra dextrose mm.	Succinic acid fed mm.	% succinic acid → glucose
Exp. 1—Mongrel terrier, male, weight 21.7 kg. Fasted for 4 days before and throughout experiment. Given 1 gm. phloridzin in oil twice daily for 3 days before and throughout experiment.								
1	1220	3.24	6.48	21.9	3.38			
2	1630	2.56	11.24	35.4	3.16			
3	1295	6.15	10.11	34.6	3.41			
4	1740	6.02	8.51	38.7	4.53	20.3	42.4	96.0
5	1920	6.14	9.56	41.2	4.32	16.7	42.4	79.3
6	2020	4.97	8.17	27.3	3.34			
7	1350	5.08	7.60	24.7	3.27			
Exp. 2—Spaniel, male, weight 16 kg. Fasted for 3 days before and throughout experiment. Given 1 gm. phloridzin in oil once daily for 2 days before and throughout experiment.								
1	1660	1.49	18.00	63.8	3.54			
2	940	2.71	14.41	51.6	3.58			
3	860	3.48	17.28	57.0	3.29			
4	980	1.32	10.30	57.2	5.54	36.4	424.0	17.2
5	650	0.60	6.58	48.0	7.28	66.6	424.0	31.5
6	620	2.63	9.68	34.6	3.57			
7	890	6.04	11.60	40.8	3.52			
Experiments of Ringer, Frankel and Jonas (*J. Biol. Chem.*, 1913, **14**, 539).								
Succinic acid given *per os*						26.0	100.0	52.0
" " " subcutaneously						47.7	100.0	95.4

by Van Slyke's method, total nitrogen by the macro-Kjeldahl method and sugar by Benedict's method. The succinic acid was fed in the free state and there was no diarrhea.

In estimating dextrose formation from a fed compound by means of the D:N ratio in the urine of the phloridzinized dog it is necessary to base your conclusions upon the highest ratio which is obtained in various experiments. On this basis our results (Exp. 1, 4th day) confirm the best observation of Ringer, *et al.*,[4] and indicate that in the phloridzinized organism succinic acid may be entirely converted to dextrose (2 mols. succinic acid = 1 mol. dextrose). However, an explanation of the relatively low percentages of conversion of succinic acid to dextrose in our Exp. 2 when large doses were fed, is necessary. This is exactly what happens when large doses of sugar are fed to the phloridzinized organism. There is no intrinsic impairment of the ability to oxidize carbohydrate in the phloridzinized dog.[5] The unnatural gradient of tissue sugar → blood sugar → urine simply reduces or prevents its oxidation. If

[5] Deuel, H. J., Jr., *J. Biol. Chem.*, 1930, **89**, 77.

enough carbohydrate or, as in this case, dextrose former is given, the gradient tissue sugar → blood sugar, becomes reversed enough to approach normal and permit an increase in dextrose oxidation. The latter is evident in Exp. 2 in the antiketogenic and nitrogen sparing effects of the succinic acid.

Summary. In the phloridzinized dog the changes in the D:N ratio indicate that small doses of succinic acid are entirely converted to dextrose (2 mols. succinic acid = 1 mol. glucose). When larger doses are given a smaller percentage is excreted in the urine as sugar but anti-ketogenic and nitrogen sparing activity indicate its conversion to dextrose before being burned.

9876 P

Atrophy of Thymus of the Rat Resulting from Administration of Adrenocorticotropic Hormone.*

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Some time ago publication was made from this laboratory of the rapid atrophy of the thymus on the administration of the gonadotropic hormone in pregnant mare serum.[1] The phenomenon was not seen in males or females after castration and was tentatively referred to the increased production of sex hormones on the part of the gonad. Selye, Browne and Collip[2] have noted atrophy of the thymus following administration of progesterone. Selye, Harlow and Collip,[3] and Schacher, Browne and Selye[4] subsequently produced regression of the thymus by administration of estrogenic, as well as androgenic substances. The preparation of adrenocorticotropic and mammotropic hormones in this laboratory has resulted in their administration under a variety of conditions. It became apparent very soon that invariable prompt reduction in size and almost

* Aided by grants from the Board of Research of the University of California and Rockefeller Foundation of New York City.

1 Evans, H. M., and Simpson, M. E., *Anat. Rec.*, 1934, **60**, 423.

2 Selye, H., Browne, J. S. L., and Collip, J. B., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 472.

3 Selye, H., Harlow, C. M., and Collip, J. B., *Endokrinologie*, 1936, **14**, Heft 1/2.

4 Schacher, J., Browne, J. S. L., and Selye, H., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 488.

complete regression of the thymus occurs[5] after administration of adrenocorticotropic hormone, while mammotropic hormone does not produce the atrophy. Although adrenocorticotropic hormone in large doses causes a stunting of growth or a loss in body weight, the doses used here which were effective in reducing thymus weight were not always associated with loss of body weight.

It is now known that an increase in the production of cortin by the adrenal is provoked by adrenocorticotropic hormone,[6] as well as a demonstrable increase in its secretion of androgenic and estrogenic substances,[7, 8, 9] and one may naturally inquire as to which of these adrenal functions we may attribute the above mentioned experimental thymic atrophy. The reduction of the thymus by adrenocorticotropic hormone with equal facility after double ovariectomy would appear to rule out the stimulation of the production of progesterone, or androgenic and estrogenic substances, from the ovary which adrenocorticotropic hormones could conceivably occasion. Since the sex hormones are known to stimulate the pituitary, which in turn could pour out any one of its secretions, it is desirable to use hypophysectomized animals to simplify explanations of hormonal effects. Hence, it would seem important to note that the thymic atrophy following administration of adrenocorticotropic hormone was secured in hypophysectomized animals. The accompanying paper shows that thymic atrophy occurs as a direct result of cortin administration.[10] This indicates that the atrophy of the thymus resulting from administration of adrenocorticotropic hormone is due in part to the increased production of cortin, but does not eliminate the possibility that increased production of male or female sex hormones in the adrenal cortex is a contributing factor.

5 Moon, H. D., PROC. SOC. EXP. BIOL. AND MED., 1936, **37**, 34.

6 Ingle, D. J., Moon, H. D., and Evans, H. M., in preparation.

7 Davidson, C. S., and Moon, H. D., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 281.

8 Davidson, C. S., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 703.

9 Moon, H. D., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 36.

10 Ingle, D. J., in preparation.

9877 P

Electrolyte Balance of the Blood in Ménière's Disease.

JOHN H. TALBOTT, MADELAINE R. BROWN, FREDERICK S. COOMBS AND WILLIAM V. CONSOLAZIO. (Introduced by H. Jackson, Jr.)

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Mygind and Dederding[1] associated the pathogenesis of Ménière's disease with a disturbance of water and salt equilibria. From their investigations they concluded that a dehydrating and an acid-forming diet was indicated in the treatment of this condition. Two years later Furstenberg, Lashmet and Lathrop[2] extended these observations, showed evidence which indicated that sodium was the offending ion and recommended a regime with a low sodium diet and intermittent periods of ammonium chloride ingestion. This regime, where employed, has been successful,[3, 4] but we believe from our data that a lowering of the sodium content of the body fluids is not its mode of action.

In this communication blood studies from 14 patients with Ménière's disease are presented. The diagnosis was confirmed by one or more clinicians and satisfied most of the criteria discussed by Crowe.[5] In all of the patients the concentration of serum total base, sodium and potassium was determined and in 4 the concentration of serum protein and hydrogen ion also. Eight patients were seen during periods of acute symptoms and bloods were taken at such times. In 6 patients, bloods were taken after the institution of a low sodium regime or during periods of freedom from symptoms after admission to the hospital. The results are given in Table I. It is observed that the concentrations of serum sodium and total fixed base are within the range for normals. The average concentration of serum sodium is slightly less in the patients with acute symptoms than in those without acute symptoms. The concentration of potassium is somewhat higher in the patients with acute symptoms.

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1 Mygind, S. H., and Dederding, D., *Acta Otolaryngologia*, 1932, **17**, 424.

2 Furstenberg, A. C., Lashmet, F. H., and Lathrop, F., *Ann. Oto. Rhin. and Laryng.*, 1934, **43**, 1035.

3 Brown, M. R., *J. A. M. A.*, 1937, **108**, 1158.

4 Furstenberg, A. C., *Transactions of the Twenty-fourth Annual Meeting of the Pacific Coast Ophthalmological Society*, 1936, **21**, 150.

5 Crowe, S. J., *Medicine*, 1938, **17**, 1.

TABLE I.

	Total fixed base	Sodium	Potassium
Concentrations are expressed in m.eq. per liter.			
Electrolytes of serum taken from 8 patients during acute symptoms.			
Max.	155.8	141.5	15.8
Min.	151.0	131.5	3.5
Aver.	153.6	138.3	6.7
Electrolytes of serum taken from 6 patients after institution of low sodium regime.			
Max.	155.8	144.8	4.7
Min.	148.0	135.0	4.4
Aver.	153.2	140.0	4.5
Range for normals as determined in this laboratory			
Max.	155	142	6
Min.	150	138	4

Four patients during symptom-free periods were given sodium salts in an attempt to precipitate vertigo, severe tinnitus or nausea. Large doses of soda bicarbonate by mouth or sodium racemic lactate by vein were used. In these patients the average concentration of serum sodium was increased from 140.8 to 143.7 m.eq. per liter, pHs from 7.40 to 7.43, and the serum water from 943 to 947 gm. per liter. None of the patients had an exacerbation of symptoms following these experiments.

From these data is is difficult to implicate the sodium ion as the offender in the precipitation of acute Ménière's disease. Since the clinical benefit of the so-called low sodium regime has been verified, a more satisfactory explanation was sought. In devising a palatable diet with a low sodium content some increase in the amount of potassium is unavoidable. It seemed reasonable, therefore, to try a high potassium intake and note the effect. For this purpose 5 patients have been placed on an average diet without restriction of sodium content but their potassium intake has been increased by the ingestion of 6 gm. of potassium chloride each day. In all of this group the clinical improvement has been comparable to that obtained with the Furstenberg regime.

In conclusion, it is believed that neither alkalosis, hydration nor a high sodium intake is associated with the pathogenesis of Ménière's disease.

9878 P

Identification of the Porphyrin Compound Found in Cultures of *C. diphtheriæ* and *mycobacteriæ*.

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Porphyrin was found by the present authors[1] in bouillon cultures of *C. diphtheriæ*, in amounts which vary with the toxicity or flocculative titer of the filtrates. Other investigators[2-6] have confirmed the occurrence of this pigment in *C. diphtheriæ* filtrates, by observations made both in the visible and in the ultraviolet spectrum. We have found a pigment, apparently identical, in Old Tuberculin, and in fluid cultures of several species of Mycobacteria. The chemical composition of the pigment, however, has remained in doubt. Its absorption-spectrum is that of a hemochromogen or metal-porphyrin compound. In ethereal extracts sharp bands are found in the visible at 574 and 538 mμ, and in the near ultraviolet an intense absorption with its maximum at 407.9 mμ. The pigment shows an oxidation-reduction change, the oxidized form being colorless. When extracts are treated with strong mineral acid the pigment breaks down, and there appears free coproporphyrin and the copper compound of this substance.

In the effort to identify the pigment we undertook the synthesis* of compounds of coproporphyrin with a number of metallic radicles: Fe, Mg, Mn, Co, Ni, Cu, Sn, and Zn. After an interruption this work has been resumed under more favorable conditions. Of the various metallic compounds studied, only those of Cu, Fe, and Zn are of present significance.

The Zn compound is formed readily when a solution of zinc salt is added to coproporphyrin in slightly alkaline aqueous solution. The combination is stable over a rather wide pH range but is disrupted

1 Coulter, C. B., and Stone, F. M., *J. Gen. Physiol.*, 1931, **14**, 583.

2 Levaditi, C., Loiseau, G., Païc, M., Phillippe, M., and Haber, P., *Compt. rendu de Soc. Biol.*, 1934, **116**, 609.

3 Wadsworth, A., Crowe, M. O'L., and Smith, L. A., *Brit. J. Exp. Path.*, 1935, **16**, 201.

4 Païc, M., *Compt. rendu de l'Acad. des Scs.*, 1935, **200**, 173.

5 Ottensooser, F., Krupski, A., and Almasy, F., *Biochem. Z.*, 1935, **277**, 314.

6 Pappenheimer, A. M., Jr., and Johnson, S. J., *Brit. J. Exp. Path.*, 1937, **18**, 239.

* A preliminary report of this work was presented at the Conference on Spectroscopy held at M.I.T. on July 18, 1934.

by strong mineral acid. The absorption-spectra in various solvents are very similar. In ethereal solution sharp bands are present about mμ 574 and 539; one of greater intensity is found at mμ 407.8. In aqueous solution the pigment exhibits an oxidation-reduction change, the oxidized form being colorless. The Zn compound thus corresponds in spectrochemical behavior with the pigment contained in culture filtrates. More complete identification is afforded by the correspondence of the fluorescence-spectrum of the pigment from culture filtrates with that of the synthetic compound. Ethereal solutions when excited by light from a carbon arc or other source of intense radiation in the near ultraviolet, give intense bands of emission at mμ 623 and 579. The fluorescence of diphtherial culture filtrates has been described very recently by Dhéré,† whose studies have been pursued independently.

The compounds of the porphyrins with copper are well-known. Copper salts combine readily with coproporphyrin, with greater avidity in acid than in alkaline reaction. The copper compound is formed when an ethereal solution of Zn coproporphyrin is treated with 5% HCl in the presence of even minute traces of Cu. It is insoluble in HCl solution, and is very stable. The intense absorption-band of the Cu compound at Å 3965 betrays its presence by a small peak in the spectrum of culture extracts which give no other evidence of it. Copper thus forms a separate compound in extracts and need not be considered a constituent of the characteristic pigment of culture filtrates. Païc has reached the same conclusion from similar evidence.

Païc has recently ascribed the strong absorption-band at Å 4080 of ethereal extracts to a ferrous compound of coproporphyrin, which he believes to be formed during the extraction of the filtrate with acetic acid ether. This explanation does not account for the fluorescence, or the visible spectrum of filtrates and ethereal extracts. Neither the ferrous nor the ferric compounds of coproporphyrin show the characteristic absorption-bands of such solutions, nor do they exhibit the phenomenon of fluorescence.

It is not possible at the present time to decide whether Zn coproporphyrin exists within the bacteria, perhaps in combination as a hemochromogen, or is formed in the culture liquid after coproporphyrin has been liberated by the disintegration of bacilli. The latter seems the more likely, but the possibility first mentioned receives support from consideration of the fluorescent emission of living *C. diphtheriae* cultures, which Dhéré and Rapetti[7] have found

† *In press.*

[7] Dhéré, Ch., and Rapetti, L., *Extrait du Bull. de l'Acad. de Méd.*, 1935, **114**, 96.

to occur in the spectral region 619 to 614 mμ. Although no fluorescent band is found within this wavelength range in *ethereal* solution of Zn coproporphyrin, we find the fluorescence bands of alkaline aqueous solution of this compound at mμ 629, 619-613, and 583.5. The most intense of these, with its maximum about 615 mμ, corresponds thus in position with the fluorescent band of the living bacteria.

9879 P

Intercellular pH Change Cannot be the Pain Factor in Ischemic Work.

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Using a capillary glass electrode in human extensor digitorum communis muscles carrying an ergograph load of 600 gm. the pH changes between the muscle fibers were determined before and after ischemic work by the method detailed in a previous publication.[1]

With the arm rendered ischemic by a brachial pressure of 160 mm. of mercury work was done until pain appeared. Simultaneously, work was stopped and pressure released. Within 10 seconds pain had disappeared, but maximal intercellular acidity was not reached before 30 to 40 seconds had elapsed. If pH continues to fall after pain ceases intercellular pH fall cannot be the cause of pain.

In another type of trial ischemic work was done until pain appeared, work was stopped and pressure released until acidity reached a plateau. Pressure was then again applied. Intercellular pH again started to fall markedly, far below the previous plateau yet the pain did not reappear. If acidity greater than that present with the pain can be created without pain under the same circumstances, then intercellular pH change cannot be the cause of the pain.

If the sensory endings responsible for ischemic pain lie within the muscle cell pH change might still be the cause of the pain, as there is no proof that intracellular and intercellular pH are identical.

[1] Maison, G. L., Orth, O. S., and Lemmer, K. E., *Am. J. Physiol.*, 1938, **121**, 311.

9880 P

Influence of Oxygen Deficiency on Reflex Dilatation of the Pupil.

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The purpose of the studies reported in this paper is twofold: 1. To extend the systematic observations of Gellhorn[1] and collaborators on the effect of oxygen deficiency on the central nervous system to the autonomic nervous system. 2. To choose a reaction which is determined exclusively or to a large extent by a central inhibitory process in order to study the influence of oxygen deficiency on such processes.

Although the literature on the problem of reflex dilatation is still controversial a number of authors have shown that this reaction is largely due to an inhibition of the parasympathetic tonus of the Edinger-Westphal nucleus. This is particularly clear from the work of Bain, Irving and McSwiney,[2] who find that the stimulation of the central end of the splanchnic fails to elicit pupillary dilatation after sectioning of the third nerve, although "section of the cervical sympathetic nerves has not been found to alter either the rate or the degree of the reaction to stimulation of somatic or visceral afferent fibers."

The experiments were carried out repeatedly on 12 rabbits, half of which had the cervical sympathetic cut on one side. The sciatic was exposed under light urethane anesthesia and 2-3 hours later the experiment was begun. The threshold reaction for pupillary dilatation was determined with faradic stimulation (Harvard inductorium, shielded electrode) while the animals inhaled air or a known O_2-N_2-mixture from Douglas bags.

Very weak currents (coil distance 11-12 cm. or at an angle) were used for 1-3 seconds and the pupillary reaction was measured with a telescope.

The results were uniform and showed that under the influence of 6-8% O_2 the threshold for pupillary dilatation was raised. This reaction was completely reversible. The same concentration of

1 Gellhorn, E., and Spiesman, I., *Am. J. Physiol.*, 1935, **112**, 519, 620, 662; Gellhorn, E., *Am. J. Physiol.*, 1936, **115**, 679; 1936, **117**, 75; Gellhorn, E., and Joslyn, A., *J. Psychol.*, 1936, **3**, 161; Gellhorn, E., *Am. J. Psychiatry*, 1937, **93**, 1413; Gellhorn, E., and Kraines, S., *Arch. Neur. a. Psych.*, 1937, **38**, 491; Gellhorn, E., and Storm, L. F. M., *Acta Oto-Laryngol.*, 1938, in press.

2 Bain, W. A., Irving, J. T., and McSwiney, B. A., *J. Physiol.*, 1935, **84**, 323.

oxygen inhaled in the presence of 5% CO_2 did not produce any significant changes in threshold. Control experiments with inhalation of 5% CO_2 in air showed no change in threshold. These experiments are in agreement with the earlier work of Gellhorn and collaborators on the effect of oxygen deficiency and CO_2 on cortical and subcortical reflexes.

The pupillary reaction on the normal and the sympathectomized side was observed independently and simultaneously by 2 observers. The threshold was the same on both sides. The humoral excitation of the pupil occurs only with far stronger and more prolonged stimulation and is distinguished from the quick reaction observed in our experiments by its longer latent period and duration. Therefore it may be concluded that the pupillary reaction under the conditions studied is due to an inhibition of the parasympathetic center of the third nerve. The experiments show that excitatory and inhibitory processes are altered in a similar way under oxygen deficiency.

9881 P

Rôle of Afferent Nerves in Response of Vasomotor Center to Oxygen Deficiency.*

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Through numerous investigations it is known that the increased respiratory response to CO_2 is due less to its effect on peripheral receptors than on the respiratory center itself, whereas a similar response to oxygen deficiency occurs only when the carotid sinus nerves are intact. The removal of the afferent fibers by elimination of all known "buffer nerves" results in a failure of respiration during O_2 deficiency (Selladurai and Wright,[1] Schmidt,[2] v. Euler and

* This work will be published in detail in the series of Medical Monographs published by the College of Medicine of the University of Illinois.

† Aided by a grant from the Graduate School of the University of Illinois to E. Gellhorn.

1 Selladurai, S., and Wright, S., *Quart. J. Exp. Physiol.*, 1932, **22**, 233.

2 Schmidt, C. F., *Am. J. Physiol.*, 1932, **102**, 94.

Liljestrand,[3] Smyth,[4] Gemmill and Reeves,[5] and Wright,[6] but *cf.* Dautrebande and Wegria[7]). Under such circumstances the blood pressure reaction to O_2 deficiency may also be converted from the rise obtained in the intact animal to a fall (Selladurai and Wright,[1] and Brewer[8]). However, the question whether the organization of the vasomotor center is similar to that of the respiratory center can only be investigated when the respiration is kept constant and thereby secondary effects of a reduced respiratory volume eliminated.

Such studies were carried out on narcotized dogs (Na-barbital, Na-amytal, chloralosane) with pneumothorax and artificial respiration. O_2-N_2 gas mixtures (1.0 to 9.8% O_2) were prepared by means of flowmeters, analyzed and inhaled from Douglas bags for periods of from 1 to 3 minutes and the blood pressure effects were recorded. After the control reaction consisting of a rise in blood pressure was obtained both vagi were cut in the neck and both carotid sinus regions were denervated. Hereafter the same O_2-N_2 mixture was inhaled and invariably an immediate fall of blood pressure was observed which occured regularly no matter whether the blood pressure level was unchanged, higher or lower than under the control conditions.

It was then attempted to show the importance of the various buffer nerves to account for this reversed reaction. Several experiments were carried out in which the effect of low oxygen on the blood pressure was studied, (1) in the normal animal, (2) after bilateral vagotomy, (3) after bilateral denervation of the carotid sinus. It was found that neither the elimination of the carotid sinus nor the bilateral vagotomy alone brought about a reversal in the blood pressure response to oxygen deficiency but that this was the case only when the two procedures were combined. The sequence was immaterial. Some experiments indicated that bilateral denervation of the carotid sinus region plus section of the nerves of Cyon reversed the blood pressure response to low oxygen, but even in these cases additional bilateral vagotomy produced a still greater fall in blood pressure.‡

[3] v. Euler, U. S., and Liljestrand, G., *Skand. Arch. Phys.*, 1936, **74**, 101.

[4] Smyth, D. H., *J. Physiol.*, 1937, **88**, 425.

[5] Gemmill, C. L., and Reeves, D. L., *Am. J. Physiol.*, 1933, **105**, 487.

[6] Wright, S., *Quart. J. Exp. Physiol.*, 1937, **26**, 63.

[7] Dautrebande, L., and Wegria, R., *Arch. int. de Phys.*, 1937, **44**, 425.

[8] Brewer, N. R., *Am. J. Physiol.*, 1937, **120**, 91.

‡ Undoubtedly, a variable number of aortic fibers are not restricted to the depressor nerve, but run in the vagus and cervical sympathetic as in the rabbit and cat (Koch, E., *Die reflektorische Selbststeuerung des Kreislaufes*, Dresden and Leipzig, 1931).

Under all the above circumstances inhalation of air containing an excess of CO_2 (4-15%) caused a rise in blood pressure and not infrequently after denervation the reaction to excess CO_2 was even increased.

Furthermore, it was ascertained that the vagus fibers which account for the maintenance of the blood pressure rise in response to low oxygen even after bilateral denervation of the carotid sinus region carry impulses from the thorax and not from the abdominal cavity. This is shown by the fact that the division of the vagi at the level of the diaphragm does not interfere with the normal blood pressure response to oxygen deficiency in a dog with both carotid sinus regions denervated.

A crucial experiment may be described briefly. A rise in blood pressure was observed after inhalation of 5.5% oxygen for 2 minutes in a normal dog, after bilateral denervation of the carotid sinus region, and after section of one vagus nerve. The significance of the remaining intact vagus was demonstrated by the observation that the cooling of these fibers produced a reversal in the response to low oxygen. When the conduction in these fibers was restored by a gradual restoration of the normal temperature the original response was reëstablished. This procedure could be repeated several times with equal success. If finally the vagus was cut, the fall in blood pressure was still greater than that observed when the nerve was blocked by cooling.

The experiments prove that the rise in blood pressure on inhalation of low oxygen is due to the effect on the chemoreceptors in the carotid sinus regions and the thorax. After these receptors are eliminated the vasomotor center is depressed by oxygen deficiency in a manner similar to that of the respiratory center. Neither the respiratory nor the vasomotor center, when investigated after exclusion of afferent fibers, react differently from cortical and subcortical centers which show in general a decrease in excitability under the influence of low oxygen.[9]

[9] Gellhorn, E., and Spiesman, I., *Am. J. Physiol.*, 1935, **112**, 519, 620, 662.

9882

Competence for Neural Plate Formation in *Hyla* and the So-called Nervous Layer of the Ectoderm.*

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The appearance of the neural plate in Amphibia is known to be dependent upon two main factors: First, there must be an inducing stimulus which normally emanates from the chorda-somite mesoderm; and secondly, the reacting ectodermal cells must be in a susceptible condition. This latter condition is now commonly referred to as "competence" for neural plate formation,[1] and has vanished by the time the egg reaches the early neurula stage.[2]

In an Anuran egg, *Hyla regilla* the competence of anterior and posterior portions of the presumptive epidermis was compared at several successive stages of development. The operative method was somewhat different from the "Einsteckungsmethode" of Mangold, in which the dorsal blastoporal lip is simply inserted into the blastocoele. In the present experiments the presumptive epidermal explant was first allowed to fuse firmly with the dorsal lip by placing the 2 pieces in contact on the floor of the operating dish. The combined explants (presumptive epidermis plus dorsal lip) were then implanted in a uniform position in another gastrula (Fig. 1). This method assures us that all the pieces of ectoderm remain in contact with the dorsal lip for an equal length of time and that they develop

TABLE I.

Summary Table of the Percentage of Neural Plates Induced in Anterior and Posterior Presumptive Epidermis by Dorsal Blastoporal Lip of the Early Gastrula.

Stage	Region of Presumptive Epidermis	No. of Eggs	No. of Neural Plates Induced	% of Inductions
Early Gastrula	Anterior	50	49	98
	Posterior	46	44	95
Mid-Gastrula	Anterior	24	19	79
	Posterior	20	7	35
Late Gastrula	Anterior	21	2	9.5
	Posterior	19	0	0
Early Neurula	Anterior	13	0	0
	Posterior	16	0	0

* Aided by a research grant from the University of California.

1 Waddington, C. H., *Philos. Trans.* B, 1932, **221**, 211.

2 Mangold, O., *Arch. f. Entw-mech.*, 1929, **117**, 586.

in the same position in the host. Close to 100% neural plate inductions are obtained *consistently* when the presumptive epidermis is from an early gastrula.

Table I shows the results obtained at several stages of development when anterior and posterior epidermis are tested.

This table includes only perfectly clear cases of neural induction in which the ectodermal cells and their nuclei were distinctly elongated and closely packed to form a neural plate or tube.

In his work on urodeles Machemer[3] did not report any definite data for the competence of anterior and posterior presumptive epidermis in the *early* gastrula stage. He makes the statement, however, that he did a few experiments on this stage and these indicated that the *posterior* presumptive epidermis was apparently more competent than the anterior (p. 450). Our results with *Hyla* (Table I) show that there is little or no difference in the capacity of anterior and posterior presumptive epidermis of the *early* gastrula. However, in the *middle*-gastrula stage, when the yolk plug measures about half the diameter of the egg, the posterior ectoderm is distinctly less competent than the anterior. This confirms Machemer's work on this stage in the Urodeles.[3] Table I further shows that when the egg has reached the advanced gastrula stage the posterior presumptive epidermis has completely lost its competence, but the anterior presumptive epidermis occasionally reacts. Presumptive epidermis from the neurula always fails to react.

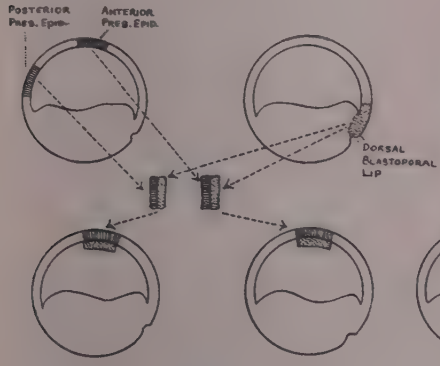

FIG. 1

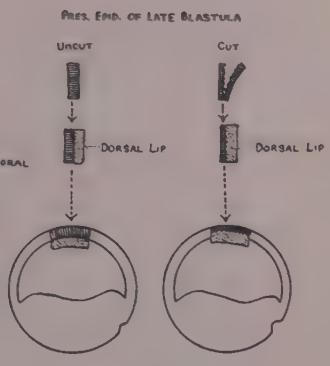

FIG. 2

Among the recent hypotheses as to the factors involved in loss of neural plate competence, is the ingenious one of Dettlaff[4] to the

[3] Machemer, H., *Arch. f. Entw-mech.*, 1932, **126**, 391.

[4] Dettlaff, T., *Zool. Jahrbücher*, Abt. allg. Zool. u. Physiol., 1936, **57**, 203.

effect that the inner layer of presumptive epidermis cells (the so-called nervous layer) gradually migrates away from the region of the presumptive epidermis toward the future neural plate. According to Dettlaff competence vanishes because it depends upon the presence of this inner layer of cells which migrates away during gastrulation. We have still another possible explanation of the loss of competence in the thinning of the ectoderm which goes hand in hand with its loss of competence. Waddington[5] had advanced evidence that mere thinning of the ectoderm plays a dominant rôle in the competence for lens; this suggests the possibility that thinning may also affect competence for neural plate formation.

The above two hypotheses were tested by the following simple experiment. In the advanced blastula, when the roof is still quite thick, a square of presumptive epidermis was removed and then cut in half *parallel* to the surface of the egg. The outer pigmented half of this explant was then combined with dorsal lip from the early gastrula and implanted into another egg (Fig. 2). Thus the inner layers of cells (the "nervous layer") stressed in Dettlaff's hypothesis were to a large extent eliminated. At the same time the piece of presumptive epidermis was reduced to about half its normal thickness. In the controls the ectoderm remained of normal thickness ("uncut," Fig. 2).

The results are perfectly clear (Table II): The thin layer of presumptive epidermis is as competent as the normal layer, as shown by the percentage of neural inductions. Furthermore, the induced neural plates develop simultaneously in both groups. Machemer[3] showed that less competent presumptive epidermis takes longer to differentiate into neural plate than the more competent.

TABLE II.
Percentage of Neural Plates Induced in Presumptive Epidermal Layers of Normal and Half-normal Thickness.

Thickness of the Ectoderm	No. of Eggs	No. Induced Neural Plates	% of Inductions
Normal	16	14	87.5
Half-normal	18	16	89

It cannot, of course, be supposed that in this experiment all the cells which constitute the so-called nervous layer of the ectoderm were removed. *It is certain, however, that a great number of these cells were removed and that the competence of the presumptive epidermis was not thereby affected.* We must conclude, therefore, that

[5] Waddington, C. H., *J. Exp. Biol.*, 1936, **13**, 86.

neither loss of the nervous layer nor mere thinning of the presumptive epidermis are responsible for its loss of competence.

Summary. 1. The anterior and posterior presumptive epidermis of the early gastrula of *Hyla regilla* possess essentially the same degree of competence for neural plate formation. 2. In the middle and late gastrula stages the posterior presumptive epidermis is less competent than the anterior. 3. Thinning of the ectoderm by removal of at least a large part of its inner layers of cells (the so-called nervous layer) in the advanced blastula stage does not impair the competence of the ectoderm.

9883 P

Influence of Vitamin D in Experimental Lead Poisoning.

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In the present investigation a study was made of the effect of the antirachitic vitamin upon the concentration of lead in the blood and upon the amount of lead deposition in the bones. This was undertaken because it has been noted previously that the severity of both experimental and clinical lead poisoning is increased under the influence of vitamin D,[1, 2, 3] or during the summer months when the sunlight is rich in ultraviolet rays.

A series of young rats, 23-26 days of age, was placed on a lead-containing diet. Half of these animals received approximately 33 Steenbock units of viosterol in halibut liver oil daily (Mead Johnson) after the first 5 days on the lead-containing diet. The composition of the diet is given in Table I. It is essentially a rickets-producing diet where 3% of $Pb(OH)_2 . 2PbCO_3$ was substituted for 3% of $CaCO_3$. At the end of the experimental period the concentration of lead in the whole blood and the amount deposited in the bones was determineed. The results of the experiments are presented in Tables II and III.

[1] Shelling, D. H., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 248; Shelling, D. H., and Hopper, K. B., *Bull. Johns Hopkins Hospital*, 1936, **58**, 193.

[2] Krehbiehl, O. F., personal communication to Dr. Shelling from Dr. C. E. Willoughby of the Chemical Experiment Station, E. I. DuPont de Nemours Company.

[3] Blackman, S. S., *Bull. Johns Hopkins Hosp.*, 1937, **61**, 1.

TABLE I.

Diet.	
70 parts	Corn Meal (Quaker Oats)
16 "	Wheat Gluten
10 "	Brewer's Yeast (Mead's)
3 "	$2(PbCO_3)Pb(OH)_2$
1 "	NaCl
Ca = 0.03 g.	per 100 g. of diet
Pb = 2.4 "	" " " " " "
= 11.6 mM.	" " " " " "
P = 0.246 g.	" " " " " "
= 7.9 mM.	" " " " " "

TABLE II.
Experiment I (Duration—22 days).

	Lead Diet	Lead Diet + Vitamin D
Number of animals	8	8
Avg. change in wt., g.	+3.3	+1.3
Avg. wt. fat free dry femurs, mg.	63.3	66.9
Avg. wt. ashed femurs, mg.	17.9	23.2
Avg. % ash of femur	28.3	34.7
Avg. lead in femurs, mg.	0.101	0.285
Avg. % lead in ashed femur	0.56	1.23

TABLE III.

	Lead Diet	Lead Diet + Vitamin D
Experiment II (Duration—34 Days).		
No. of animals	5	8
Avg. change in wt., g.	+1.4	—0.8
Avg. wt. of lead in tibia, mg.	0.18	0.30
Avg. lead in tibia, %	0.34	0.57
Avg. lead in whole blood, mg. per 100 cc.	1.25	2.13
Experiment III (Duration—23 Days).		
No. of animals	11	10
*Avg. change in wt., g.	—1.6	—3.0
Avg. lead in whole blood,	0.65	1.04
Mg. per 100 cc.	0.71	1.30
Ca, mg. %	8.9	8.7
P, mg. %	3.1	6.2
Ca × P	27.6	54.0

*Divided into two groups.

From these results, a definite influence of the antirachitic vitamin may be readily observed. Both the lead concentration in the blood and the amount of lead present in the bones was about twice as high in the vitamin-fed animals as in those not receiving this addition. These findings help to explain the previously observed increased severity of lead poisoning under the influence of vitamin D or ultraviolet rays, for with increased lead levels in the blood, one usually finds more acute symptoms of plumbism.

The relation of higher concentrations of lead in the blood to in-

creased deposition in the bones is not wholly unexpected. Such phenomena are observed in the deposition of inorganic bone normally. As a rule, the higher the concentration of the bone forming elements in the blood plasma the greater the ease of deposition. Lead in this case may be considered as one of the bone-forming elements, *i. e.*, a part of the inorganic matter of bone, although not a normal one. The mechanism of deposition of lead salts seems to follow the phenomena observed with the normal bone-forming constituents, namely, calcium and phosphate, *i. e.*, the higher the value of the product formed by multiplying the concentrations of these two constituents in the blood serum, the greater the ease of deposition. Another parallel worth mentioning is that vitamin D, as a rule, is instrumental in raising the above product and thereby promotes the normal formation of inorganic bone, just as it promotes deposition of lead in the bone.

Conclusions. Vitamin D causes a rise in the concentration of lead in the blood stream and in the bones of rats suffering from lead poisoning.

9884 P

Influence of Dietary Calcium and Phosphorus upon Action of Vitamin D in Experimental Lead Poisoning.

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In the preceding paper[1] a marked effect of vitamin D on the blood and bone lead concentrations of lead-fed animals was demonstrated. In view of the great importance attached to the dietary calcium and phosphorus content in lead poisoning[2, 3, 4] investigations were un-

1 Sobel, A. E., Gawron, O., and Kramer, B. K., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 433.

2 Shelling, D. H., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 248; Shelling, D. H., *The Parathyroids in Health and in Disease*, St. Louis, 1935, C. V. Mosby Company; Shelling, D. H., and Hopper, K. B., *Bull. Johns Hopkins, Hosp.*, 1936, **58**, 137.

3 Gray, I., *J. Am. Med. Assn.*, 1935, **104**, 200.

4 Aub, J. C., *J. Am. Med. Assn.*, 1935, **104**, 87; Aub, J. C., Fairhall, L. T., Minot, A. S., and Reznikoff, P., *Lead Poisoning-Medicine Monographs*, Baltimore, Williams and Wilkins Co., 1926, 7.

dertaken to determine the part these factors play upon the previously noted action of the antirachitic vitamin.

The basal diet used in the previous experiment contained traces of calcium (0.03%) and small amounts of phosphorus (0.246%) to which basic lead carbonate was added. To this basal diet 2.5% of calcium carbonate was added to produce a high calcium low phosphorus diet; and 2.75% of anhydrous Na_2HPO_4 was added to produce the high phosphorus low calcium diet. The diets are described below:

		Parts
Basal Diet:	Yellow corn meal (Quaker Oats)	70
	Wheat gluten	16
	Brewer's Yeast (Mead Johnson)	10
	NaCl	1
Diet A:	Basal diet + 3 parts of $Pb(OH)_2 \cdot 2PbCO_3$	
	Pb = 2.4 % = 11.6 mg. mol./100 g.	
	Ca = 0.03 % = .8 " " "	
	P = 0.246% = 7.9 " " "	
Diet B:	Basal diet + 0.5 part of $Pb(OH)_2 \cdot 2PbCO_3$ + 2.5 parts of $CaCO_3$	
	Pb = 0.4 % = 1.93 mg. mol./100 g.	
	Ca = 1.0 % = 25.0 " " "	
	P = 0.246% = 7.9 " " "	
Diet C:	Basal diet + 1.5 parts of $Pb(OH)_2 \cdot 2PbCO_3$ + 2.75 Na_2HPO_4	
	Pb = 1.2 % = 5.8 mg. mol./100 g.	
	Ca = 0.03 % = .8 " " "	
	P = 0.846% = 27.2 " " "	

Albino rats raised in our laboratory from an original Wistar strain were used. The mothers were kept on the stock diet of Bills, *et al.*[5] The young were weaned at 21 days, at which time they were placed on the stock diet. At the age of 23 to 25 days this was replaced by the experimental diets described above. One half of each group was given 33 Steenbock units of vitamin D. (Mead Johnson's 250 D-viosterol in halibut liver oil, diluted in maize oil.) After 23 to 25 days the animals were sacrificed. The lead content of the blood was determined by the method of Willoughby, *et al.*[6] The lead content of the femora was determined by a modification of the above method (to be described).

The results of the experiments are presented in Table I. The vitamin D groups contained a higher percentage of lead in both the dried bone and the bone ash. Parallel effects were noted in the blood lead concentration of groups A and B. In group C which received the high phosphorus low calcium diet, the concentration of

[5] Bills, C. E., Honeywell, E. M., Wirick, A. M., and Nussmeier, M. J., *J. Biol. Chem.*, 1931, **90**, 619.

[6] Willoughby, C. E., Kraemer, E. O., and Smith, F. L., *Ind. Eng. Chem.*, Anal. Ed., 1935, **7**, 33.

TABLE I.
Influence of Dietary Calcium and Phosphorus upon Action of Vitamin D in Experimental Lead Poisoning.

	Group A		Group B		Group C	
	Diet A	Diet A + Vit. D	Diet B	Diet B + Vit. D	Diet C	Diet C + Vit. D
No. of animals	8	8	4	4	5	5
Avg. change in wt., g.	+3.3	+1.3	+21.0	+9.0	+12	+7
Avg. ash of fat free femora, %	28.3	34.7	26.5	40.4	40.0	35.7
Avg. Pb in fat free femora, mg. per 100 g.	160	326	73.3	216.8	115.3	305.2
Avg. Pb in bone ash, mg. per 100 g.	565	1230	291	542	292	778
Avg. Pb in whole blood, mg. per 100 cc.	0.71	1.3	0.22	0.68	>0.1	>0.1

lead in the blood was too low to be accurately determined. It is worthwhile to note, however, that even though this group received 3 times as much lead in the diet as the high calcium low phosphorus group, the blood lead concentrations in group C are distinctly below those of group B. The beneficial effects of the high phosphorus diet in experimental and clinical lead poisoning[2, 3] may be thus explained in terms of lowered blood lead concentration. Furthermore, in spite of the much lower blood lead concentration in group C as compared to group B, slightly more lead was deposited in the bones of the former group. To elucidate this apparent contradiction more knowledge is required of the thermodynamic activity of lead ions and phosphate ions in the blood stream. It is hoped that further investigations, which are in progress, will throw additional light upon the subject.